U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-257 588

Applied Ecology Seminar Training Manual

Environmental Protection Agency

Nov 72

BIBLIOGRAPHIC DATA SHEET

1. Report No.

2.

3. Recipient's Accession No. PB-257 588

4. Title and Subtitle

Applied Ecology Seminar.
Training Manual

5. Report Date Nov 72

6.

7. Author(s)

8. Performing Organization Rept. No.

9. Performing Organization Name and Address

Environmental Protection Agency
Washington, D. C.
Office of Water Programs

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Address

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstracts

Contents:
The nature and meaning of pollution; Biological techniques in water quality investigations; Analysis and use of biological data; Legal and Jurisdictional aspects of water quality.

17. Key Words and Document Analysis. 17a. Descriptors

*Meetings,
*Ecology,
*Water pollution,
Aquatic biology,
Definitions,
Bioassay,
Indicator species,
Plankton,
Bacteriology,
Aquatic microbiology,
Toxicology,
Water quality,
Data,
Evaluation,
Methodology,
Manuals,
Law(jurisprudence).

17b. Identifiers/Open-Ended Terms

Aquatic ecosystems,
Periphyton.

17c. COSATI Field/Group 6 F 13 B

18. Availability Statement

National Technical Information Service
Springfield, Virginia 22161

19. Security Class (This Report) UNCLASSIFIED

20. Security Class (This Page) UNCLASSIFIED

21. No. of Pages 434p

22. Price 11.75/2.25

FORM NTIS-35 (REV. 10-73) ENDORSED BY ANSI AND UNESCO. THIS FORM MAY BE REPRODUCED USCOMM-DC 8265-P74

PB 257 588

APPLIED ECOLOGY SEMINAR

TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAMS

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

APPLIED ECOLOGY SEMINAR

This course is intended for technically oriented persons concerned with planning, administering, collecting, evaluating, interpreting and reporting biological data related to water quality studies in both fresh and marine waters.

ENVIRONMENTAL PROTECTION AGENCY
Office of Water Programs
TRAINING PROGRAM

November 1972

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Programs, Environmental Protection Agency.

Due to the limited availability of the manuals, it is not appropriate to cite them as technical references in bibliographies or other forms of publication.

References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Office of Water Programs, Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

Joseph Bahnick

Joseph Bahnick
Acting Chief
Direct Technical Training Branch
Division of Manpower and Training
Office of Water Programs
Environmental Protection Agency

TRAINING PROGRAM

Through the Office of Water Program, Environmental Protection Agency conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

Training is available at five installations of the Agency. These are: the National Training Center located at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio; the Robert S. Kerr Water Research Center, Ada, Oklahoma; the Southeast Water Laboratory, Athens, Georgia; the Pacific Northwest Water Laboratory, Corvallis, Oregon; and the Hudson-Delaware Basins Office, Edison, New Jersey.

The objectives of the Training Program are to provide specialized training in the field of water pollution control which will lead to rapid application of new research findings through updating of skills of technical and professional personnel, and to train new employees recruited from other professional or technical areas in the special skills required. Increasing attention is being given to development of special courses providing an overview of the nature, causes, prevention, and control of water pollution.

Scientists, engineers, and recognized authorities from other Agency programs, from other government agencies, universities, and industry supplement the training staff by serving as guest lecturers. Most training is conducted in the form of short-term courses of one or two weeks' duration. Subject matter includes selected practical features of plant operation and design, and water quality evaluation in field and laboratory. Specialized aspects and recent developments of sanitary engineering, chemistry, aquatic biology, microbiology, and field and laboratory techniques not generally available elsewhere, are included.

The primary role and the responsibility of the States in the training of wastewater treatment plant operators are recognized. Technical support of operator-training programs of the States is available through technical consultations in the planning and development of operator-training courses. Guest appearances of instructors from the Environmental Protection Agency, and the loan of instructional materials such as lesson plans and visual training aids, may be available through special arrangement. These training aids, including reference training manuals, may be reproduced freely by the states for their own training programs. Special categories of training for personnel engaged in treatment plant operations may be developed and made available to the States for their own further production and presentation.

An annual Bulletin of Courses is prepared and distributed by the Office of Water Programs. The Bulletin includes descriptions of courses, schedules, application blanks, and other appropriate information. Organizations and interested individuals not on the mailing list should request a copy from one of the training centers mentioned above.

U.S. Environmental Protection Agency

OFFICE OF WATER PROGRAMS

MANPOWER DEVELOPMENT STAFF

R. F. Guay, Director

Academic Training Branch
State and Local Operator Training Programs
Office of Environmental Activities

Direct Technical Training Branch

National Training Center
Cincinnati, OH 45268

REGIONAL MANPOWER OFFICES

REGION I

Manpower Development Branch
Division of Air and Water Programs
424 Trapelo Road
Waltham, MA 02514

REGION II

Manpower Development and Training Office
Air and Water Programs
26 Federal Plaza
New York, NY 10007

REGION III

Manpower Development Office
Air and Water Programs
Curtis Building
6th and Walnut Streets
Philadelphia, PA 19106

REGION IV

Manpower Development Branch
Division of Air and Water Programs
1421 Peachtree Street, NE, Fourth Floor
Atlanta, GA 30309

REGION V

Manpower Development Branch
Office of Air and Water Programs
1 N. Wacker Drive
Chicago, IL 60606

REGION VI

Manpower Development Branch
Air and Water Programs Division
1600 Patterson
Dallas, TX 75201

REGION VII

Manpower Development Branch
Air and Water Programs
1735 Baltimore
Kansas City, MO 64108

REGION VIII

Manpower Development Branch
Air and Water Division
1860 Lincoln Street - 9th Floor
Denver, CO 80203

REGION IX

Manpower Development Branch
Air and Water Division
100 California Street
San Francisco, CA 94111

REGION X

Manpower and Training Branch
Division of Air and Water Programs
1200 Sixth Avenue - Mail Stop 345
Seattle, WA 98101

7.11.72

APPLIED ECOLOGY SEMINAR (146)

3 days

DALLAS, TEXAS November 14-16, 1972

This seminar is intended for technically oriented persons concerned with planning, administering, collecting, evaluating, interpreting and reporting biological data related to water quality studies in both fresh and marine waters.

The main objective of this seminar is to promote more effective utilization of biological techniques which are fundamental in water pollution control. The student will develop a more complete understanding of the advantages and potential contributions of biological data and investigations to administrators, project leaders and others. Biologists will gain a better understanding of the limitations and restrictions placed on the administrator, and the nature of biological data which will be most useful for the improvement of water quality.

The following topics will usually be included:

Orientation in the nature of the aquatic environment
and aquatic ecology

The nature of biological parameters

Definition of "pollution" under various circumstances

Orientations in the nature of various biological procedures
and habitats such as taxonomy, benthos, periphyton,
plankton, nekton, bioassays, and bacteriological
investigations, followed by searching discussion of
the potential of each in water quality enhancement

Techniques for the evaluation and interpretation of
biological data

The multilateral problems of communication

The use of biological data in the establishment and
administration of water quality standards and
criteria

Much of the seminar will consist of short formal presentations followed by extended informal discussions. Laboratory or field sessions usually are not scheduled, although an optional field excursion following the formal course may be arranged on occasion. The program may be adjusted for from one to five days depending on objectives. Three days are recommended. Adjustments may also be made as to the relative emphasis on fresh or salt water or both.

CONTENTS

Title or Description **Outline Number**

SECTION A INTRODUCTION

Man and the Ecosystem A- 1
The Aquatic Environment A- 2
Biological Parameters A- 3
Aquatic Organisms of Significance in Pollution Surveys A- 4
Global Deterioration and our Environmental Crisis A- 5

SECTION B THE NATURE AND MEANING OF POLLUTION

Mechanisms of Pollution Tolerance and Sensitivity B- 1
Effects of Pollution on Aquatic Life B- 2
Nutrients: The Basis of Productivity B- 3
Biological Aspects of Natural Self Purification B- 4
The Effects of Pollution on Lakes B- 5
Significance of Eutrophication B- 6
Pollution of the Marine Environment B- 7
Water Temperature and Water Quality B- 8
Biotic Effects of Siltation B- 9
The Cycling of Radionuclides in the Aquatic Environment B-10
Bacteria and Protozoa as Toxicological Indicators B-11
Pathogenic Microorganisms in Water and Sewage B-12
Effects of Pollution on Fish B-13
Influence of Land Runoff on Water Quality B-14
Ground Water Quality Studies B-15
The Effects of Organisms on Pollution and the Environment B-16
Effects and Control of Oil Pollution B-17

Title or Description — Outline Number

SECTION C BIOLOGICAL TECHNIQUES IN WATER QUALITY INVESTIGATIONS

Techniques for the Biological Evaluation of Pollutants C- 1

Field Investigations

Biological Field Methods C- 2

The Identification of Organisms

Critical Problems in Systematics C- 3
The System of Biological Classification C- 4

Plankton Studies

Techniques of Plankton Analysis C- 5
Determination of Plankton Productivity C- 6

Artificial Substrates

Artificial Substrates C- 7
Artificial and Related Substrates - References C- 8

Fish Kills

Procedures for Fish Kill Investigations C- 9

Human Health

Bacteriological Indicators of Water Pollution C-10

Evaluation of Toxicity

Bioassay and Biomonitoring C-11

Bottom Organisms

Using Benthic Biota in Water Quality Evaluations C-12

Periphyton

Attached Growths (Periphyton or Aufwuchs) C-13
Stream Invertebrate Drift C-14

Title or Description	Outline Number
SECTION D ANALYSIS AND USE OF BIOLOGICAL DATA	
The Interpretation of Biological Data with Reference to Water Quality	D- 1
Significance and Interpretation of Biological Data	D- 2
Presentation of Data	D- 3
Organization and Presentation of Biological Data and Reports	D- 4
Preparation of Survey Reports	D- 5
An Initiation into Statistics	D- 6
Administrative Need for Biological Information	D- 7
Dissemination of Technical Information to the Public	D- 8
Water Quality Surveys	D- 9
Application of Biological Data	D-10
SECTION E LEGAL AND JURISDICTIONAL ASPECTS OF WATER QUALITY	
Case Preparation and Courtroom Procedure	E- 1
Water Quality Criteria	E- 2
Research Needs: Fish, Other Aquatic Life and Wildlife	E- 3

SECTION A

INTRODUCTION

	Outline Number
Man and the Ecosystem	A-1
The Aquatic Environment	A-2
Biological Parameters	A-3
Aquatic Organisms of Significance in Pollution Surveys	A-4
Global Deterioration and our Environmental Crisis	A-5

MAN AND THE ECOSYSTEM

I INTRODUCTION: HOW SMART IS MAN?

A "The most important problem facing human beings today is the problem of atomic war or, in biological terms, the problem of extinction. Ironically, the next most important problem is the problem of over-population."

B "....man has been using his power to alter his environment - with a blind pursuit of immediate objectives and a disregard of secondary effects, that could endanger the very existence of life on this planet."

C "The concern is not with nature alone, but with the total relation between man and the world around him. Its object is not just man's welfare, but the dignity of man's spirit."

D The need for clear definition and effective solution of environmental problems is urgent, as society in this 20th Century heads into large scale problems of over-population, environmental pollution, and multiple-use resource management.

II THE NATURE OF AN ECOSYSTEM

A An ecosystem is a relatively independent and complete system of life such as might be found in and around a lake, a continent or the earth.

B Three general types of organisms may be defined: consumers, producers, and reducers.

1 Producers perform the original selection and synthesis of nutrient mineral materials into living substance. Energy (usually solar) is stored during synthesis (photosynthesis) and released in respiration.

2 Consumers depend on the producers for food. They are the animals. Many successive stages of food transfer may be described (trophic or energy levels, food chains, food webs). Much of the energy stored by the producers is released in the processes of digestion, assimilation, and respiration.

3 Reducers are the bacteria and other fungi which finally return organic matter to its original mineral condition, releasing any stored energy remaining in the processes of respiration.

4 Organically stored energy may also be released physically by burning (oxidation).

C These processes are inevitable and inescapable. Time is of no consequence.

III ECOLOGICAL RELATIONSHIPS OF MAN

A A great number of ecological principles and concepts are involved in describing and explaining the earth and its ecosystems.

1 A climax association of organisms is one which tends to remain stable over an extended period of time, pending changes in physical and chemical environmental factors. Climaxes are generally dominated by one or a few species or types, e.g., grass, trees.

2 Climax communities may be temporarily upset or even destroyed by transient catastrophies such as fire, flood, or pestilence. They will in (geologic) time, however, become re-established.

3 A pest (-ilence) which destroys its host or habitat destroys itself.

B There is interaction between the organism and its (total) environment; e. g., the worm and the soil, man and his food web (the nutritional interrelationships of a community).

1 Since Pleistocene times man has occupied a describable role in his ecosystem. His influence has been growing in recent millennia.

2 At one time the races of men were strictly local. Now man is global in distribution.

3 The nature of an organism's environmental effect is a result of its characteristics: The clam plows the bottom, the locust devours, man thinks.

C Man is thus the first organism on earth to be able to analyze and regulate its environmental interactions.

1 Technological progress is virtually synonomous with greater:

Consumption of natural resources
Production of goods
Production of people
Pollution of the environment

2 Because of his insight into the consequences of his behavior, man has the theoretical capacity to so regulate his activities that he can eventually live in a balanced environment. How smart is man?

IV CONCLUSIONS

A Man is no more and no less than an organism interacting with its environment.

B Since man is a thoughtful being, it behooves him to desist from destroying his own environment, lest he go down in geologic history as the cataclysmic factor of the post Pleistocene.

REFERENCES

1 USDI The Population Challenge. Yearbook No. 2. 1966.

2 Markert, C. L. Biological Limits of Population Growth. Bioscience 16 (12). December 1966.

3 Hesse, Richard, Allen, W. C., and Schmidt, K. P. Ecological Animal Geography. John Wiley & Sons, Inc. New York. 1937.

4 Harrar, J. G. Food, Science, and People. Trans. N. Y. Acad. Sci., Ser. II, 20 (3):263-277. January 1958.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, EPA, Cincinnati, OH 45268.

THE AQUATIC ENVIRONMENT

Part 1: The Nature and Behavior of Water

I INTRODUCTION

The earth is physically divisible into the lithosphere or land masses, and the hydrosphere which includes the oceans, lakes, streams, and subterranean waters.

A Upon the hydrosphere are based a number of sciences which represent different approaches. Hydrology is the general science of water itself with its various special fields such as hydrography, hydraulics, etc. These in turn merge into physical chemistry and chemistry.

B Limnology and oceanography combine aspects of all of these, and deal not only with the physical liquid water and its various naturally occurring solutions and forms, but also with living organisms and the infinite interactions that occur between them and their environment.

C Water quality management, including pollution control, thus looks to all branches of aquatic science in efforts to coordinate and improve man's relationship with his aquatic environment.

II SOME FACTS ABOUT WATER

A Water is the only abundant liquid on our planet. It has many properties most unusual for liquids, upon which depend most of the familiar aspects of the world about us as we know it.

TABLE 1

UNIQUE PROPERTIES OF WATER

Property	Significance
Highest heat capacity (specific heat) of any solid or liquid (except NH_3)	Stabilizes temperatures of organisms and geographical regions
Highest latent heat of fusion (except NH_3)	Thermostatic effect at freezing point
Highest heat of evaporation of any substance	Important in heat and water transfer of atmosphere
The only substance that has its maximum density as a liquid (4°C)	Fresh and brackish waters have maximum density above freezing point. This is important in vertical circulation pattern in lakes.
Highest surface tension of any liquid	Controls surface and drop phenomena, important in cellular physiology
Dissolves more substances in greater quantity than any other liquid	Makes complex biological system possible. Important for transportation of materials in solution.
Pure water has the highest di-electric constant of any liquid	Leads to high dissociation of inorganic substances in solution
Very little electrolytic dissociation	Neutral, yet contains both H+ and OH^- ions
Relatively transparent	Absorbs much energy in infra red and ultra violet ranges, but little in visible range. Hence "colorless"

B Physical Factors of Significance

1 Water substance

Water is not simply "H_2O" but in reality is a mixture of some 33 different substances involving three isotopes each of hydrogen and oxygen (ordinary hydrogen H^1, deuterium H^2, and tritium H^3; ordinary oxygen O^{16}, oxygen 17, and oxygen 18) plus 15 known types of ions. The molecules of a water mass tend to associate themselves as polymers rather than to remain as discrete units. (See Figure 1)

2 Density

a Temperature and density: Ice. Water is the only known substance in which the solid state will float on the liquid state. (See Table 2)

SUBSTANCE OF WATER

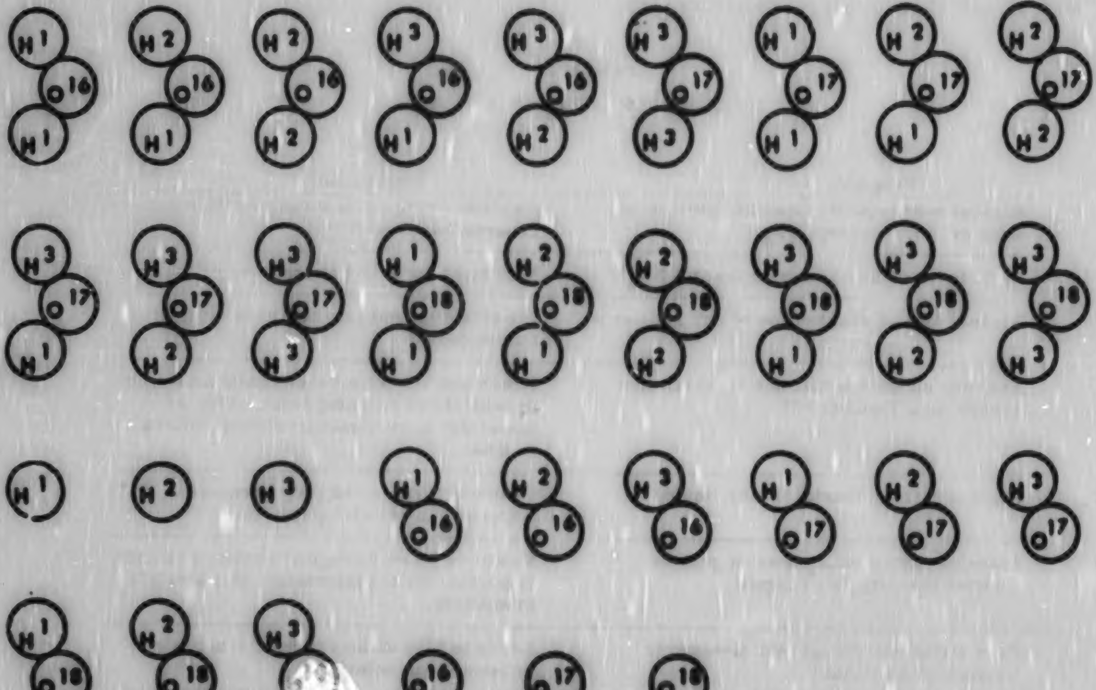

H1 O16 H1
H2 O16 H1
H2 O16 H2
H3 O16 H1
H3 O16 H2
H3 O17 H3
H1 O17 H1
H2 O17 H1
H2 O17 H2
H3 O17 H1
H3 O17 H2
H3 O17 H3
H1 O18 H1
H2 O18 H1
H2 O18 H2
H3 O18 H1
H3 O18 H2
H3 O18 H3
H1
H2
H3
H1 O16
H2 O16
H3 O16
H1 O17
H2 O17
H3 O17
H1 O18
H2 O18
H3 O18
O16
O17
O18

Figure 1

TABLE 2

EFFECTS OF TEMPERATURE ON DENSITY OF PURE WATER AND ICE*

Temperature (° C)	Density	
	Water	Ice **
-10	.99815	.9397
- 8	.99869	.9360
- 6	.99912	.9020
- 4	.99945	.9277
- 2	.99970	.9229
0	.99987----	.9168
2	.99997	
4	1.00000	
6	.00997	
8	.00988	
10	.00973	

* Tabular values for density, etc., represent statistical estimates by various workers rather than absolute values, due to the variability of water.

** Regular ice is known as "ice I". Four or more other "forms" of ice are known to exist (ice II, ice III, etc.), having densities at 1 atm. pressure ranging from 1.1595 to 1.67. These are of extremely restricted occurrence and may be ignored in most routine operations.

This ensures that ice usually forms on top of a body of water and tends to insulate the remaining water mass from further loss of heat. Did ice sink, there could be little or no carryover of aquatic life from season to season in the higher latitudes. Frazil or needle ice forms colloidally at a few thousandths of a degree below 0° C. It is adhesive and may build up on submerged objects as "anchor ice", but it is still typical ice.

1) Seasonal increase in solar radiation annually warms surface waters in summer while other factors result in winter cooling. The density differences resulting establish two classic layers: the epilimnion or surface layer, and the hypolimnion or lower layer, and in between is the thermocline or shear-plane.

2) While for certain theoretical purposes a thermocline is defined as a zone in which the temperature changes one degree centigrade for each meter of depth, in practice, any transitional layer between two relatively stable masses of water of different temperatures (and probably other qualities too) may be regarded as a thermocline.

3) Obviously the greater the temperature differences between epilimnion and hypolimnion and the sharper the gradient in the thermocline, the more stable will the situation be.

4) From information given above, it should be evident that while the temperature of the hypolimnion rarely drops much below 4° C, the epilimnion may range from 0° C upward.

5) It should also be emphasized that when epilimnion and hypolimnion achieve the same temperature, stratification no longer exists, and the entire body of water behaves hydrologically as a unit, and tends to assume uniform chemical and physical characteristics. Such periods are called overturns and

usually result in considerable water quality changes of physical, chemical, and biological significance.

6) When stratification is present, however, each layer behaves relatively independently, and considerable quality differences may develop.

7) Thermal stratification as described above has no reference to the size of the water mass; it is found in oceans and puddles.

8) The relative densities of the various isotopes of water also influence its molecular composition. For example, the lighter O_{16} tends to go off first in the process of evaporation, leading to the relative enrichment of air by O_{16} and the enrichment of water by O_{17} and O_{18}. This can lead to a measurably higher O_{18} content in warmer climates. Also, the temperature of water in past geologic ages can be closely estimated from the ratio of O_{18} in the carbonate of mollusc shells.

b Dissolved and/or suspended solids may also affect the density of natural waters.

TABLE 3
EFFECTS OF DISSOLVED SOLIDS ON DENSITY

Dissolved Solids (Grams per liter)	Density (at 4° C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35 (mean for sea water)	1.02822

c Density caused stratification

1) Density differences produce stratification which may be permanent, transient, or seasonal.

2) Permanent stratification exists for example where there is a heavy mass of brine in the deeper areas of a basin which does not respond to seasonal or other changing conditions.

3) Transient stratification may occur with the recurrent influx of tidal water in an estuary for example, or the occasional influx of cold muddy water into a deep lake or reservoir.

4) Seasonal stratification involves the annual establishment of the epilimnion, hypolimnion, and thermocline as described above. The spring and fall overturns of such waters materially affect biological productivity.

5) Density stratification is not limited to two-layered systems; three, four, or even more layers may be encountered in larger bodies of water.

d A "plunge line" may develop at the mouth of a stream. Heavier water flowing into a lake or reservoir plunges below the lighter water mass of the epiliminium to flow along at a lower level. Such a line is usually marked by an accumulation of floating debris.

2 The viscosity of water is greater at lower temperatures (see Table 4). This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action. Living organisms more easily support themselves in the more viscous (and also denser) cold waters of the arctic than in the less viscous warm tropical waters.

TABLE 4

VISCOSITY OF WATER (In millipoises at 1 atm)

Temp. ° C	Dissolved solids in g/L			
	0	5	10	30
-10	26.0	----	----	----
- 5	21.4	----	----	----
0	17.94	18.1	18.24	18.7
5	15.19	15.3	15.5	16.0
10	13.10	13.2	13.4	13.8
30	8.00	8.1	8.2	8.6
100	2.84	----	----	----

3 Surface tension has biological as well as physical significance. Organisms whose body surfaces cannot be wet by water can either ride on the surface film or in some instances may be "trapped" on the surface film and be unable to re-enter the water.

4 Incident solar radiation is the prime source of energy for virtually all organic and most inorganic processes on earth. For the earth as a whole, the total amount (of energy) received annually must exactly balance that lost by reflection and radiation into space if climatic and related conditions are to remain relatively constant over geologic time.

a For a given body of water, immediate sources of energy include in addition to solar irradiation: terrestrial heat, transformation of kinetic energy (wave and current action) to heat, chemical and biochemical reactions, convection from the atmosphere, and condensation of water vapor.

b The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water, thus tending to warm the upper layers.

5 Water movements

a Waves or rhythmic movement

The best known are traveling waves caused by wind. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

Standing waves or seiches occur in lakes, estuaries, and other enclosed bodies of water, but are seldom large enough to be observed. An "internal wave or seich" is an oscillation in a submersed mass of water such as a hypolimnion, accompanied by compensating oscillation in the overlying water such that no significant change in surface level is detected. Shifts in submerged water masses of this type can have severe effects on the biota and also on human water uses where withdrawals are confined to a given depth. Descriptions and analyses of many other types and sub-types of waves and wave-like movements may be found in the literature.

b Tides

Tides are the longest waves known in the ocean, and are evident along the coast by the rhythmic rise and fall of the water. While part and parcel of the same phenomenon, it is often convenient to refer to the rise and fall of the water level as "tide", and to the accompanying currents as "tidal currents".

Tides are basically caused by the attraction of the sun and moon on water masses, large and small; however, it is only in the oceans and certain of the larger lakes that true tidal action has been demonstrated. The patterns of tidal action are enormously complicated by local topography, interaction with seiches, and other factors. The literature on tides is very large.

c Currents (except tidal currents) are steady a rhythmic water movements which have had major study only in oceanography although they are best known from rivers and streams. They primarily are concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or terrestrial topography. Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

d Coriolis force is a result of interaction between the rotation of the earth, and the movement of masses or bodies on the earth. The net result is a slight tendency for moving objects to veer to the right in the northern hemisphere, and to the left in the southern hemisphere. While the result in fresh waters is usually negligible, it may be considerable in marine waters. For example, other factors permitting, there is a tendency in estuaries for fresh waters to move toward the ocean faster along the right bank, while salt tidal waters tend to intrude farther inland along the left bank. Effects are even more dramatic in the open oceans.

e Langmuir circulation (or L. spirals) is the interlocking rotation of somewhat cylindrical masses of surface water under the influence of wind action. The axes of the cylinders are parallel to the direction of the wind.

To somewhat oversimplify the concept, a series of adjoining cells might be thought of as chains of interlocking gears in which at every other contact the teeth are rising while at the alternate contacts, they are sinking (Figure 2).

The result is elongated masses of waste rising or sinking together. This produces the familiar "wind rows" of foam, flotsam and jetsam, or plankton often seen streaking windblown lakes or oceans. Certain zoo-plankton struggling to maintain a position near the surface often collect in the down current between two Langmuir cells, causing such an area to be called the "red dance", while the clear upwelling water between is the "blue dance".

This phenomenon may be important in water or plankton sampling on a windy day.

6 The pH of pure water has been determined between 5.7 and 7.01 by various workers. The latter value is most widely accepted at the present time. Natural waters of course vary widely according to circumstances.

C The elements of hydrology mentioned above represent a selection of some of the more conspicuous physical factors involved in working with water quality. Other items not specifically mentioned include: molecular structure of waters, interaction of water and radiation, internal pressure, acoustical characteristics, pressure-volume-temperature relationships, refractivity, luminescence, color, dielectrical characteristics and phenomena, solubility, action and interactions of gases, liquids and solids, water vapor, ices, phenomena of hydrostatics and hydrodynamics in general.

REFERENCES

1 Buswell, A.M. and Rodebush, W.H. Water. Sci. Am. April 1956.

2 Dorsey, N. Ernest. Properties of Ordinary Water - Substance. Reinhold Publ. Corp. New York. pp. 1-673. 1940.

3 Hutcheson, George E. A Treatise on Limnology. John Wiley Company. 1957.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

THE AQUATIC ENVIRONMENT

Part 2: The Aquatic Environment as an Ecosystem

I INTRODUCTION

Part 1 introduced the lithosphere and the hydrosphere. Part 2 will deal with certain general aspects of the biosphere, or the sphere of life on this earth, which photographs from space have shown is a finite globe in infinite space.

This is the habitat of man and the other organisms. His relationships with the aquatic biosphere are our common concern.

II THE BIOLOGICAL NATURE OF THE WORLD WE LIVE IN

A We can only imagine what this world must have been like before there was life.

B The world as we know it is largely shaped by the forces of life.

1 Primitive forms of life created organic matter and established soil.

2 Plants cover the lands and enormously influence the forces of erosion.

3 The nature and rate of erosion affect the redistribution of materials (and mass) on the surface of the earth (topographic changes).

4 Organisms tie up vast quantities of certain chemicals, such as carbon and oxygen.

5 Respiration of plants and animals releases carbon dioxide to the atmosphere in influential quantities.

6 CO_2 affects the heat transmission of the atmosphere.

C Organisms respond to and in turn affect their environment. Man is one of the most influential.

III ECOLOGY IS THE STUDY OF THE INTERRELATIONSHIPS BETWEEN ORGANISMS, AND BETWEEN ORGANISMS AND THEIR ENVIRONMENT.

A The ecosystem is the basic functional unit of ecology. Any area of nature that includes living organisms and nonliving substances interacting to produce an exchange of materials between the living and nonliving parts constitutes and ecosystem. (Odum, 1959)

1 From a structural standpoint, it is convenient to recognize four constituents as composing an ecosystem (Figure 1).

a Abiotic NUTRIENT NUMERALS which are the physical stuff of which living protoplasm will be synthesized.

b Autotrophic (self-nourishing) or PRODUCER organisms. These are largely the green plants (holophytes), but other minor groups must also be included (See Figure 2). They assimilate the nutrient minerals, by the use of considerable energy, combine them into living organic substance.

c Heterotrophic (other-nourishing) CONSUMERS (holozoic), are chiefly the animals. They ingest (or eat) and digest organic matter, releasing considerable energy in the process.

d Heterotrophic REDUCERS are chiefly bacterial and fungi that return complex organic compounds back to the original abiotic mineral condition, thereby releasing the remaining chemical energy.

2 From a functional standpoint, an ecosystem has two parts (Figure 2)

Preceding page blank

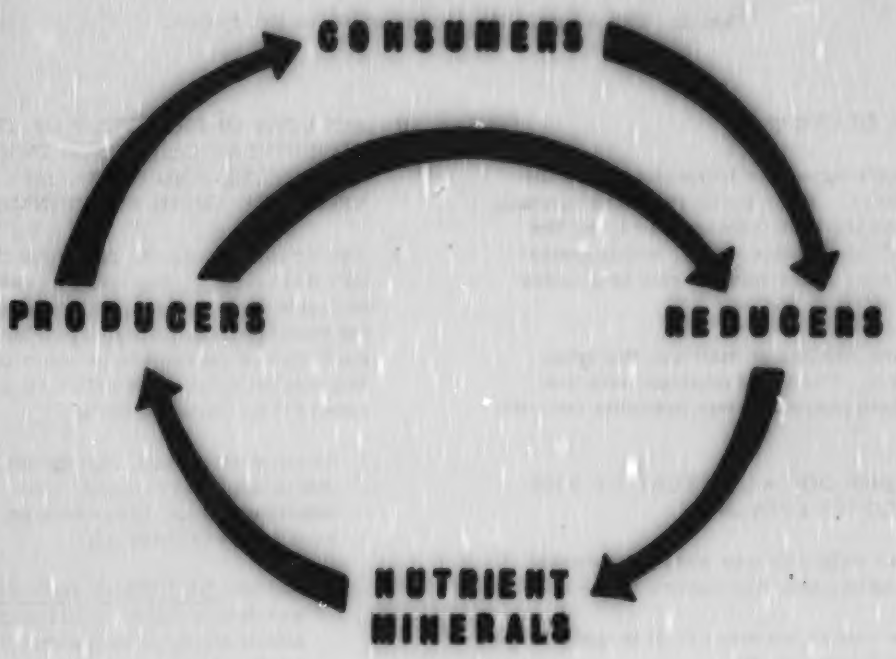


FIGURE 1

a The autotrophic or producer organisms, which construct organic substance.

b The heterotrophic or consumer and reducer organisms which destroy organic substance.

3 Unless the autotrophic and heterotrophic phases of the cycle approximate a dynamic equilibrium, the ecosystem and the environment will change.

B Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms. (Figure 2)

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal subgroups can be defined on the basis of relative complexity of structure.

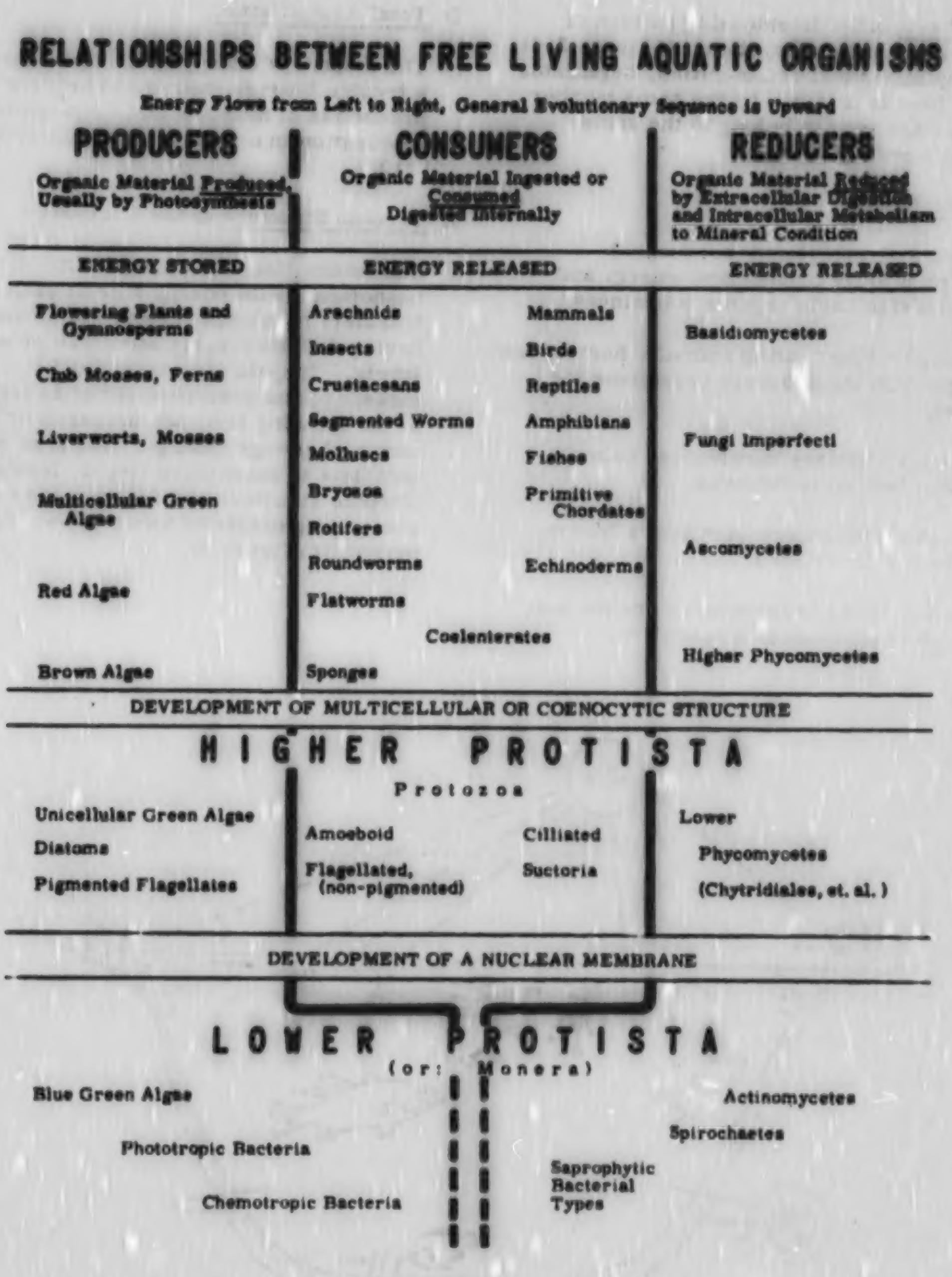
a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the higher protista.

C Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

IV FUNCTIONING OF THE ECOSYSTEM

A A food chain is the transfer of food energy from plants through a series of organisms with repeated eating and being eaten. Food chains are not isolated sequences but are interconnected.


RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS
Energy Flows from Left to Right, General Evolutionary Sequence is Upward
PRODUCERS
Organic Material Produced, Usually by Photosynthesis
CONSUMERS
Organic Material Ingested or Consumed Digested Internally
REDUCERS
Organic Material Reduced by Extracellular Digestion and Intracellular Metabolism to Mineral Condition
ENERGY STORED
ENERGY RELEASED
ENERGY RELEASED
Flowering Plants and Gymnosperms
Club Mosses, Ferns
Liverworts, Mosses
Multicellular Green Algae
Red Algae
Brown Algae
Arachnids
Insects
Crustaceans
Segmented Worms
Molluscs
Bryozoa
Rotifers
Roundworms
Flatworms
Coelenterates
Sponges
Mammals
Birds
Reptiles
Amphibians
Fishes
Primitive Chordates
Echinoderms
Basidiomycetes
Fungi Imperfecti
Ascomycetes
Higher Phycomycetes
DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC STRUCTURE
HIGHER PROTISTA
Protozoa
Unicellular Green Algae
Diatoms
Pigmented Flagellates
Amoeboid
Flagellated, (non-pigmented)
Ciliated
Suctoria
Lower Phycomycetes (Chytridiales, et. al.)
DEVELOPMENT OF A NUCLEAR MEMBRANE
LOWER PROTISTA
(or: Monera)
Blue Green Algae
Phototropic Bacteria
Chemotropic Bacteria
Saprophytic Bacterial Types
Spirochaetes
Actinomycetes

BI. ECO. pl. 2a. 1. 69

FIGURE 2

B **A food web** is the interlocking pattern of food chains in an ecosystem. (Figures 3, 4) In complex natural communities, organisms whose food is obtained by the same number of steps are said to belong to the same trophic (feeding) level.

C **Trophic Levels**

1 First - Green plants (producers) (Figure 5) fix biochemical energy and synthesize basic organic substances.

2 Second - Plant eating animals (herbivores) depend on the producer organisms for food.

3 Third - Primary carnivores, animals which feed on herbivores.

4 Fourth - Secondary carnivores feed on primary carnivores.

5 Last - Ultimate carnivores are the last or ultimate level of consumers.

D **Total Assimilation**

The amount of energy which flows through a trophic level is distributed between the production of biomass and the demands of respiration in a ratio of approximately 1:10.

E **Trophic Structure** of the Ecosystem

The interaction of the food chain phenomena (with energy loss at each transfer) results in various communities having definite trophic structure or energy levels. Trophic structure may be measured and described either in terms of the standing crop per unit area or in terms of energy fixed per unit area per unit time at successive trophic levels. Trophic structure and function can be shown graphically by means of ecological pyramids (Figure 5).

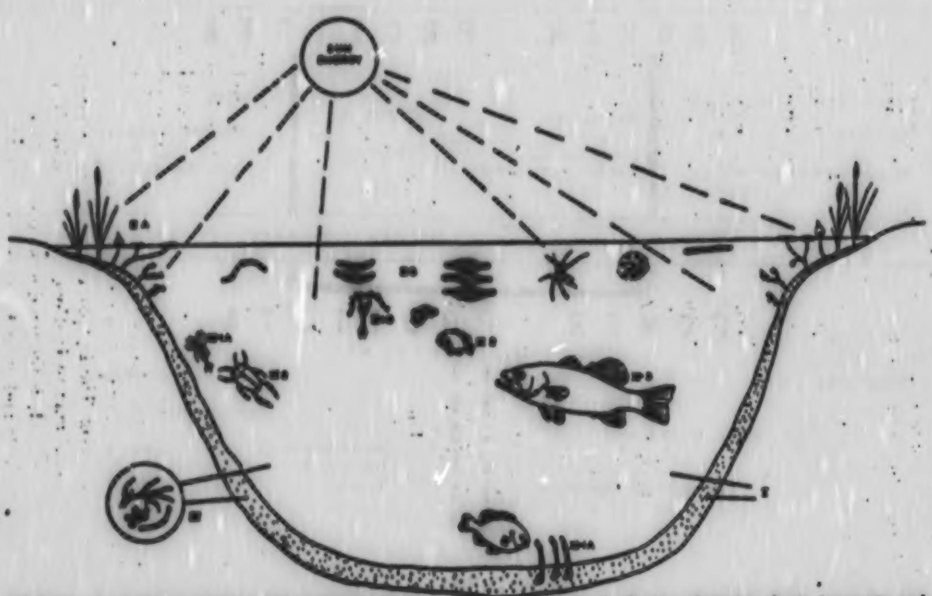


Figure 3. Diagram of the pond ecosystem. Basic units are as follows: I, abiotic substances—basic inorganic and organic compounds; IIA, producers—rooted vegetation; IIB, producers—phytoplankton; III-1A, primary consumers (herbivores)—bottom forms; III-1B, primary consumers (herbivores)—zooplankton; III-2, secondary consumers (carnivores); III-3, tertiary consumers (secondary carnivores); IV, decomposers—bacteria and fungi of decay.

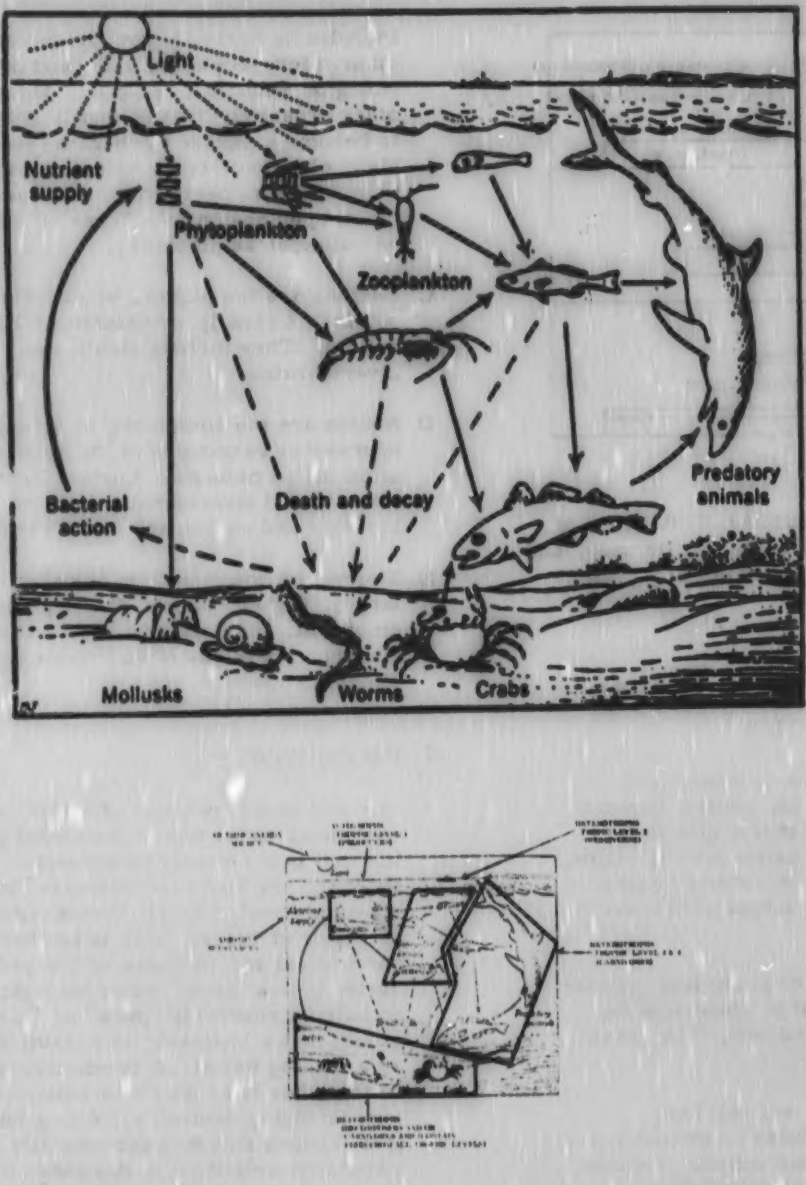

Light
Nutrient supply
Phytoplankton
Zooplankton
Bacterial action
Death and decay
Predatory animals
Mollusks
Worms
Crabs

Figure 4. A MARINE ECOSYSTEM (After Clark, 1954 and Patten, 1966)

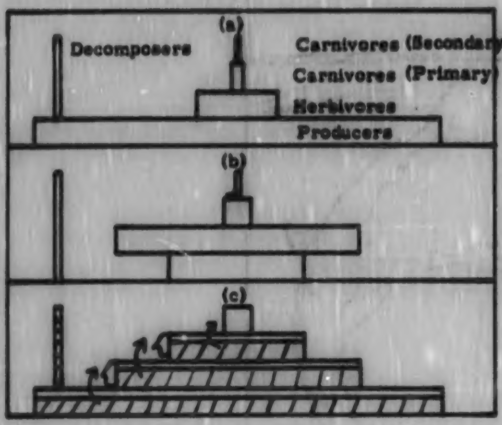


Figure 5. HYPOTHETICAL PYRAMIDS of (a) Numbers of individuals, (b) Biomass, and (c) Energy (Shading Indicates Energy Loss).

V BIOTIC COMMUNITIES

A Plankton are the macroscopic and microscopic animals, plants, bacteria, etc., floating free in the open water. Many clog filters, cause tastes, odors, and other troubles in water supplies. Eggs and larvae of larger forms are often present.

1 Phytoplankton are plant-like. These are the dominant producers of the waters, fresh and salt, "the grass of the seas".

2 Zooplankton are animal-like. Includes many different animal types, range in size from minute protozoa to gigantic marine jellyfishes.

B Periphyton (or Aufwuchs) - The communities of microscopic organisms associated with submerged surfaces of any type or depth. Includes bacteria, algae, protozoa, and other microscopic animals, and often the young or embryonic stages of algae and other organisms that normally grow up to become a part of the benthos (see below). Many planktonic types will also adhere to surfaces as periphyton, and some typical periphyton may break off and be collected as plankters.

C Benthos are the plants and animals living on, in, or closely associated with the bottom. They include plants and invertebrates.

D Nekton are the community of strong aggressive swimmers of the open waters, often called pellagic. Certain fishes, whales, and invertebrates such as shrimps and squids are included here.

E The marsh community is based on larger "higher" plants, floating and emergent. Both marine and freshwater marshes are areas of enormous biological production.

VI PRODUCTIVITY

A The biological resultant of all physical and chemical factors is the quantity of life that may actually be present. The ability to produce this "biomass" is often referred to as the "productivity" of a body of water. This is neither good nor bad per se. A water of low productivity is a "poor" water biologically, and also a relatively "pure" or "clean" water; hence desirable as a water supply or a bathing beach. A productive water on the other hand may be a nuisance to man or highly desirable. It is a nuisance if foul odors and/or weed-chocked waterways result, it is desirable if bumper crops of bass, catfish, or oysters are produced. Open oceans on the other hand have a very low level of productivity in general.

REFERENCES

1 Clarke, G. L. Elements of Ecology. John Wiley & Sons, New York. 1954.

2 Cooke, W. B. Trickling Filter Ecology. Ecology 40(2):273-291. 1959.

3 Hanson, E. D. Animal Diversity. Prentice-Hall, Inc., New Jersey. 1964.

4 Hedgpeth, J. W. Aspects of the Estuarine Ecosystem. Amer. Fish. Soc., Spec. Publ. No. 3. 1966.

5 Odum, E. P. Fundamentals of Ecology. W. B. Saunders Company, Philadelphia and London. 1959.

6 Patten, B. C. Systems Ecology. Bio-Science. 16(9). 1966.

7 Whittaker, R. H. New Concepts of Kingdoms. Science 163:150-160. 1969.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45268.

THE AQUATIC ENVIRONMENT

Part 3. The Freshwater Environment

I INTRODUCTION

The freshwater environment as considered herein refers to those inland waters not detectably diluted by ocean waters, although the lower portions of rivers are subject to certain tidal flow effects.

Certain atypical inland waters such as saline or alkaline lakes, springs, etc., are not treated, as the main objective is typical inland water.

All waters have certain basic biological cycles and types of interactions most of which have already been presented. Hence this outline will concentrate on aspects essentially peculiar to fresh inland waters.

II PRESENT WATER QUALITY AS A FUNCTION OF THE EVOLUTION OF FRESH WATERS

A The history of a body of water determines its present condition. Natural waters have evolved in the course of geologic time into what we know today.

B Streams

In the course of their evolution, streams in general pass through four general stages of development which may be called: birth, youth, maturity, and old age.

1 Establishment or birth. In an extant stream, this might be a "dry run" or headwater stream-bed, before it had eroded down to the level of ground water.

2 Youthful streams; when the stream-bed is eroded below the ground water level, spring water enters and the stream becomes permanent.

3 Mature streams; have wide valleys, a developed flood plain, deeper, more turbid, and usually warmer water, sand, mud, silt, or clay bottom materials which shift with increase in flow.

4 In old age, streams have approached geologic base level. During flood stage they scour their beds and deposit materials on the flood plain which may be very broad and flat. During normal flow the channel is refilled and many shifting bars are developed. (Under the influence of man this pattern may be broken up, or temporarily interrupted. Thus an essentially "youthful" stream might take on some of the characteristics of a "mature" stream following soil erosion, organic enrichment, and increased surface runoff. Correction of these conditions might likewise be followed by at least a partial reversion to the "original" condition).

C Lakes and Reservoirs

Geological factors which significantly affect the nature of either a stream or lake include the following:

1 The geographical location of the drainage basin or watershed.

2 The size and shape of the drainage basin.

3 The general topography, i.e., mountainous or plains.

4 The character of the bedrocks and soils.

5 The character, amount, annual distribution, and rate of precipitation.

Preceding page blank.

6 The natural vegetative cover of the land is of course responsible to many of the above factors and is also severely subject to the whims of civilization. This is one of the major factors determining runoff versus soil absorption, etc.

D Lakes have a developmental history which somewhat parallels that of streams.

1 The method of formation greatly influences the character and subsequent history of lakes.

2 Maturing or natural eutrophication of lakes.

a If not already present shoal areas are developed through erosion of the shore by wave action and undertow.

b Currents produce bars across bays and thus cut off irregular areas.

c Silt brought in by tributary streams settles out in the quiet lake water.

d Rooted aquatic plants grow on shoals and bars, and in doing so cut off bays and contribute to the filling of the lake.

e Dissolved carbonates and other materials are precipitated in the deeper portions of the lake in part through the action of plants.

f When filling is well advanced, mats of sphagnum moss may extend outward from the shore. These mats are followed by sedges and grasses which finally convert the lake into a marsh.

3 Extinction of lakes. After lakes reach maturity, their progress toward filling up is accelerated. They become extinct through:

a The downcutting of the outlet.

b Filling with detritus eroded from the shores or brought in by tributary streams.

c Filling by the accumulation of the remains of vegetable materials growing in the lake itself.
(Often two or three processes may act concurrently)

III PRODUCTIVITY IN FRESH WATERS

A Fresh waters in general and under natural conditions by definition have a lesser supply of dissolved substances than marine waters, and thus a lesser basic potential for the growth of aquatic organisms. By the same token, they may be said to be more sensitive to the addition of extraneous materials (pollutants, nutrients, etc.) The following notes are directed toward natural geological and other environmental factors as they affect the productivity of fresh waters.

B Factors Affecting Stream Productivity (See Table 1)

TABLE 1

EFFECT OF SUBSTRATE ON STREAM PRODUCTIVITY*

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Marl	6
Fine Gravel	9
Gravel and silt	14
Coarse gravel	32
Moss on fine gravel	89
Fissidens (moss) on coarse gravel	111
Ranunculus (water buttercup)	194
Watercress	301
Anacharis (waterweed)	452

*Selected from Tarzwell 1937

To be productive of aquatic life, a stream must provide adequate nutrients, light, a suitable temperature, and time for growth to take place.

1 Youthful streams, especially on rock or sand substrates are low in essential nutrients. Temperatures in mountainous regions are usually low, and due to the steep gradient, time for growth is short. Although ample light is available, growth of true plankton is thus greatly limited.

2 As the stream flows toward a more "mature" condition, nutrients tend to accumulate, and gradient diminishes and so time of flow increases, temperature tends to increase, and plankton flourish.

Should a heavy load of inert silt develop on the other hand, the turbidity would reduce the light penetration and consequently the general plankton production would diminish.

3 As the stream approaches base level (old age) and the time available for plankton growth increases, the balance between turbidity, nutrient levels, and temperature and other seasonal conditions, determines the overall productivity.

C Factors Affecting the Productivity of Lakes

1 The size, shape, and depth of the lake basin. Shallow water is more productive than deeper water since more light will reach the bottom to stimulate rooted plant growth. As a corollary, lakes with more shoreline, having more shallow water, are in general more productive. Broad shallow lakes and reservoirs have the greatest production potential (and hence should be avoided for water supplies).

2 Hard waters are generally more productive than soft waters as there are more plant nutrient minerals available. This is often greatly influenced by the character of the soil and rocks in the watershed and the quality and quantity of ground water entering the lake. In general, pH ranges of 6.8 to 8.2 appear to be most productive.

TABLE 2

EFFECT OF SUBSTRATE ON LAKE PRODUCTIVITY *

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Pebbles	4
Clay	8
Flat rubble	9
Block rubble	11
Shelving rock	77

*Selected from Tarzwell 1937

3 Turbidity reduces productivity as light penetration is reduced.

4 The presence or absence of thermal stratification with its semi-annual turnovers affects productivity by distributing nutrients throughout the water mass.

5 Climate, temperature, prevalence of ice and snow, are also of course important.

D Factors Affecting the Productivity of Reservoirs

1 The productivity of reservoirs is governed by much the same principles as that of lakes, with the difference that the water level is much more under the control of man. Fluctuations in water level can be used to deliberately increase or decrease productivity. This can be demonstrated by a comparison of the TVA reservoirs which practice a summer drawdown with some of those in the west where a winter drawdown is the rule.

2 The level at which water is removed from a reservoir is important to the productivity of the stream below.

The hypolimnion may be anaerobic while the epilimnion is aerobic, for example, or the epilimnion is poor in nutrients while the hypolimnion is relatively rich.

3 Reservoir discharges also profoundly affect the DO, temperature, and turbidity in the stream below a dam. Too much fluctuation in flow may permit sections of the stream to dry, or provide inadequate dilution for toxic waste.

VII CLASSIFICATION OF LAKES AND RESERVOIRS

A The productivity of lakes and impoundments is such a conspicuous feature that it is often used as a convenient means of classification.

1 Oligotrophic lakes are the younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes in their deeper waters.

2 Eutrophic lakes are more mature, more turbid, and richer. They are usually shallower. They are richer in dissolved solids; N, P, and Ca are abundant. Plankton is abundant and there is often a rich bottom fauna.

3 Dystrophic lakes, such as bog lakes, are low in pH, water yellow to brown, dissolved solids, N, P, and Ca scanty but humic materials abundant, bottom fauna and plankton poor, and fish species are limited.

B Reservoirs may also be classified as storage and run of the river.

1 Storage reservoirs have a large volume in relation to their inflow.

2 Run of the river reservoirs have a large flow-through in relation to their storage value.

C According to location, lakes and reservoirs may be classified as polar, temperate, or tropical. Differences in climatic and geographic conditions result in differences in their biology.

VIII SUMMARY

A A body of water such as a lake, stream, or estuary represents an intricately balanced system in a state of dynamic equilibrium. Modification imposed at one point in the system automatically results in compensatory adjustments at associated points.

B The more thorough our knowledge of the entire system, the better we can judge where to impose control measures to achieve a desired result.

REFERENCES

1 Chamberlin, Thomas C. and Salisburg, Rollin P. Geological Processes and Their Results. Geology 1: pp i-xix, and 1-654. Henry Holt and Company. New York. 1904.

2 Frey, David G. Limnology in North America. Univ. Wisc. Press. 1963.

3 Hutcheson, George E. A Treatise on Limnology Vol. I Geography, Physics and Chemistry. 1957. Vol. II. Introduction to Lake Biology and the Limnoplankton. 1115 pp. 1967. John Wiley Co.

4 Hynes, H.B.N. The Ecology of Running Waters. Univ. Toronto Press. 555 pp. 1970.

5 Ruttner, Franz. Fundamentals of Limnology. University of Toronto Press. pp. 1-242. 1953.

6 Tarzwell, Clarence M. Experimental Evidence on the Value of Trout 1937 Stream Improvement in Michigan. American Fisheries Society Trans. 66:177-187. 1936.

7 U.S. Dept. of Health, Education, and Welfare. Public Health Service. Algae and Metropolitan Wastes. Transactions of a seminar held April 27-29, 1960 at the Robert A. Taft Sanitary Engineering Center. Cincinnati, OH. No. SEC TR W61-3.

8 Ward and Whipple. Fresh Water Biology. (Introduction). John Wiley Company. 1918.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45268.

THE AQUATIC ENVIRONMENT

Part 4. The Marine Environment and its Role in the Total Aquatic Environment

I INTRODUCTION

A The marine environment is arbitrarily defined as the water mass extending beyond the continental land masses, including the plants and animals harbored within. This water mass is large and deep, covering about 70 percent of the earth's surface and being as deep as 7 miles. The salt content averages about 35 parts per thousand. Life extends to all depths.

B The general nature of the water cycle on earth is well known. Because the relatively large surface area of the earth is covered with water, roughly 70 percent of the earth's rainfall is on the seas. (Figure 1)

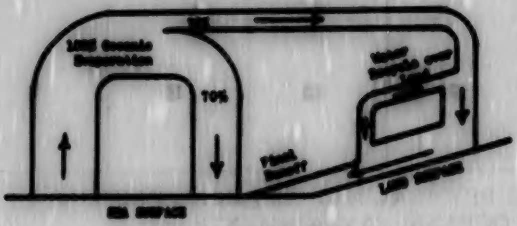


Figure 1. THE WATER CYCLE

Since roughly one third of the earth's rain which falls on the land is again recycled through the stratosphere (see Figure 1 again), the total amount of water washing over the earth's surface is significantly greater than one third of the total world rainfall. It is thus not surprising to note that the rivers which finally empty into the sea carry a considerable burden of dissolved and suspended solids picked up from the land. This is the substance of geological erosion. (Table 1)

TABLE 1

PERCENTAGE COMPOSITION OF THE MAJOR IONS OF TWO STREAMS AND SEA WATER

(Data from Clark, F.W., 1924, "The Composition of River and Lake Waters of the United States", U.S. Geol. Surv., Prof. Paper No. 135; Harvey, H.W., 1957, "The Chemistry and Fertility of Sea Waters", Cambridge University Press, Cambridge)

Ion	Delaware River at Lambertville, N.J.	Rio Grande at Laredo, Texas	Sea Water
Na	6.70	14.78	30.4
K	1.46	.85	1.1
Ca	17.49	13.73	1.16
Mg	4.81	3.03	3.7
Cl	4.23	21.65	55.2
SO_4	17.49	30.10	7.7
CO_3	32.95	11.55	*HCO_3 0.35

C For this presentation, the marine environment will be (1) described using an ecological approach, (2) characterized ecologically by comparing it with freshwater and estuarine environments, and (3) considered as a functional ecological system (ecosystem).

II FRESHWATER, ESTUARINE, AND MARINE ENVIRONMENTS

Distinct differences are found in physical, chemical, and biotic factors in going from a freshwater to an oceanic environment. In general, environmental factors are more constant in freshwater (river) and oceanic environments when compared to the highly variable and harsh environments of estuarine and coastal waters.

A Physical and Chemical Factors (Figure 2)

1 Rivers

2 Estuary and coastal waters

3 Oceans

Preceding page blank

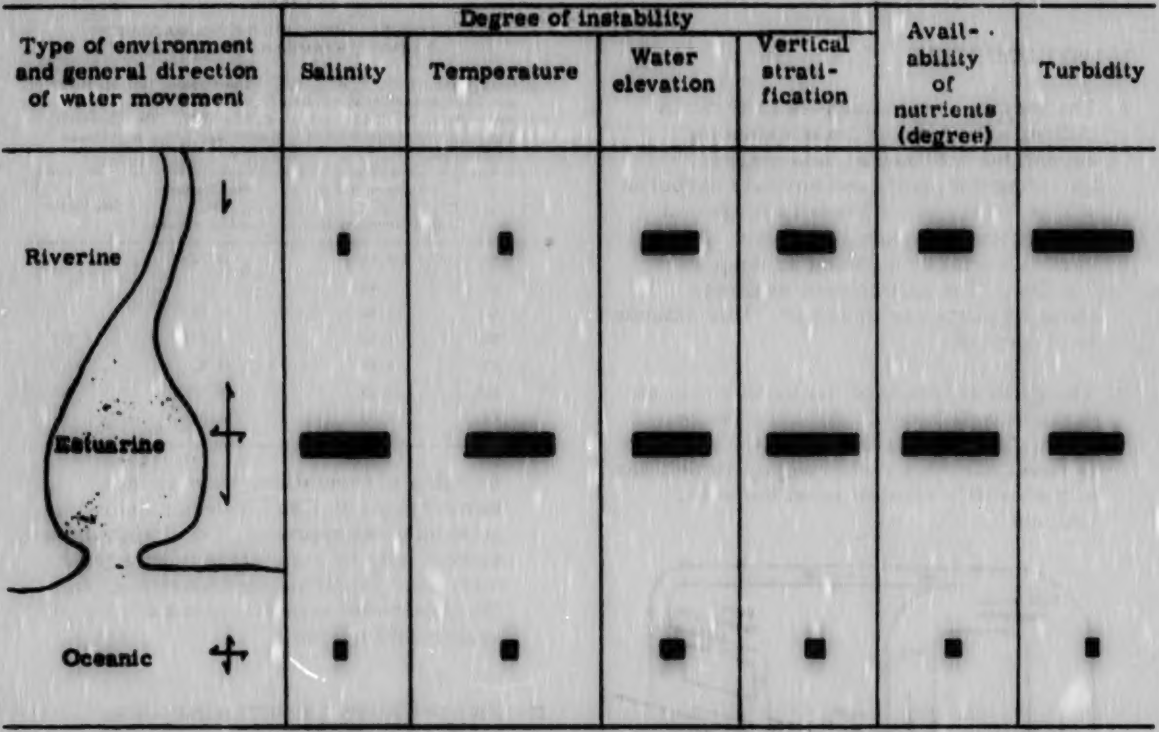


Figure 2 . RELATIVE VALUES OF VARIOUS PHYSICAL AND CHEMICAL FACTORS FOR RIVER, ESTUARINE, AND OCEANIC ENVIRONMENTS

B Biotic Factors

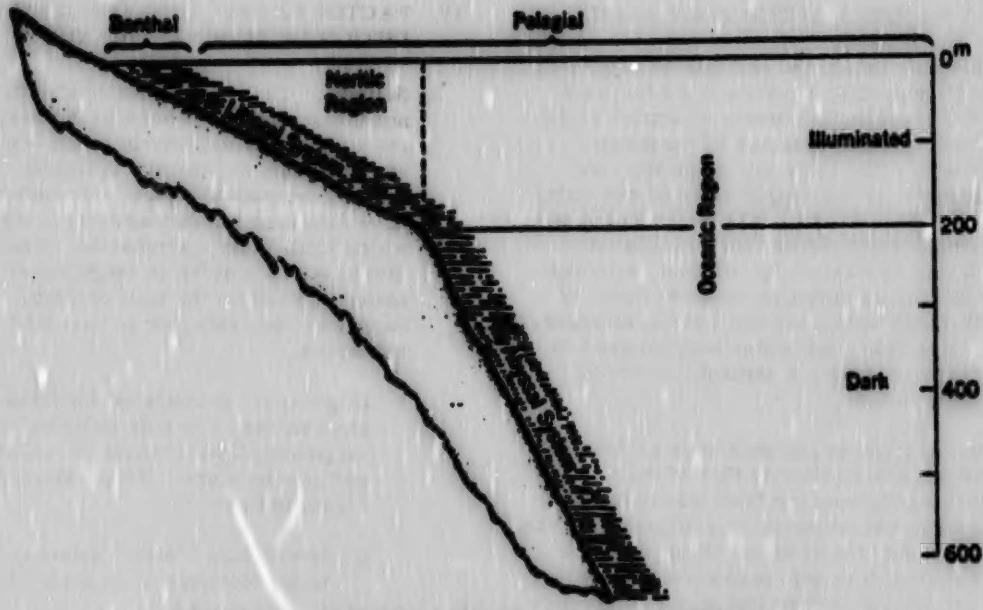
1 A complex of physical and chemical factors determine the biotic composition of an environment. In general, the number of species in a highly variable environment tends to be less than the number in a more stable environment (Hedgpeth, 1966).

2 The dominant animal species (in terms of total biomass) which occur in estuaries are often transient, spending only a part of their lives in the estuaries. This results in better utilization of a rich environment.

C Zones of the Sea

The nearshore environment is often classified in relation to tide level and water depth. The nearshore and oceanic regions together are often classified in relation to light penetration and water depth.

1 Neritic - Relatively shallow-water zone which extends from the high-tide mark to the edge of the continental shelf. (Figure 3)



Primary subdivisions of the marine habitat.

Figure 3.

a Stability of physical factors is intermediate between estuarine and oceanic environments.

b Phytoplankters are the dominant producers but in some locations attached algae are also important as producers.

c The animal consumers are zooplankton, nekton, and benthic forms.

2 Oceanic - The region of the ocean beyond the continental shelf. Divided into three parts, all relatively poorly populated compared to the neritic zone.

a Euphotic zone - Waters into which sunlight penetrates (often to the bottom in the neritic zone). The zone of basic productivity. Often extends to 600 feet below the surface.

1) Physical factors fluctuate less than in the neritic zone.

2) Producers are the phytoplankton and consumers are the zooplankton and nekton.

b Bathyal zone - From the bottom of the euphotic zone to about 6,000 feet.

1) Physical factors relatively constant but light is absent.

2) Producers are absent and consumers are scarce.

c Abyssal zone - All the sea below the bathyal zone.

1) Physical factors more constant than in bathyal zone.

2) Producers absent and consumers not as abundant as in the bathyal zone.

III SEA WATER AND THE BODY FLUIDS

A Sea water is a most suitable environment for living cells, because it contains all of the chemical elements essential to the growth and maintenance of plants and animals. The ratio and often the concentration of the major salts of sea water are strikingly similar in the cytoplasma and body fluids of marine organisms. This similarity is also evident, although modified somewhat in the body fluids of both fresh water and terrestrial animals. For example, sea water may be used in emergencies as a substitute for blood plasma in man.

B Since marine organisms have an internal salt content similar to that of their surrounding medium (isotonic condition) osmoregulation poses no problem. On the other hand, fresh water organisms are hypertonic (osmotic pressure of body fluids is higher than that of the surrounding water). Hence, fresh water animals must constantly expend more energy to keep water out (i.e., high osmotic pressure fluids contain more salts, the action being then to dilute this concentration with more water).

1 Generally, marine invertebrates are narrowly poikilosmotic, i.e., the salt concentration of the body fluids changes with that of the external medium. This has special significance in estuarine situations where salt concentrations of the water often vary considerably in short periods of time.

2 Marine bony fish (teleosts) have lower salt content internally than externally (hypotonic). In order to prevent dehydration, water is ingested and salts are excreted through special cells in the gills.

IV FACTORS AFFECTING THE DISTRIBUTION OF MARINE ORGANISMS

A Salinity - The concentration of salts is not the same everywhere in the sea; in the open ocean salinity is much less variable than in the ever changing estuary or coastal water. Organisms have different tolerances to salinity which limit their distribution. The distributions may be in large water masses, such as the Gulf Stream, Sargasso Sea, etc., or in bays and estuaries.

1 In general, animals in the estuarine environment are able to withstand large and rapid changes in salinity and temperature. These animals are classified as:

a Euryhaline ("eury" meaning wide) - wide tolerance to salinity changes.

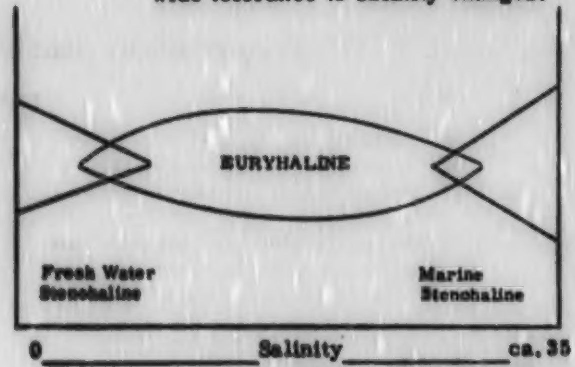


Figure 4. Salinity Tolerance of Organisms

b Eurythermal - wide tolerance to temperature changes.

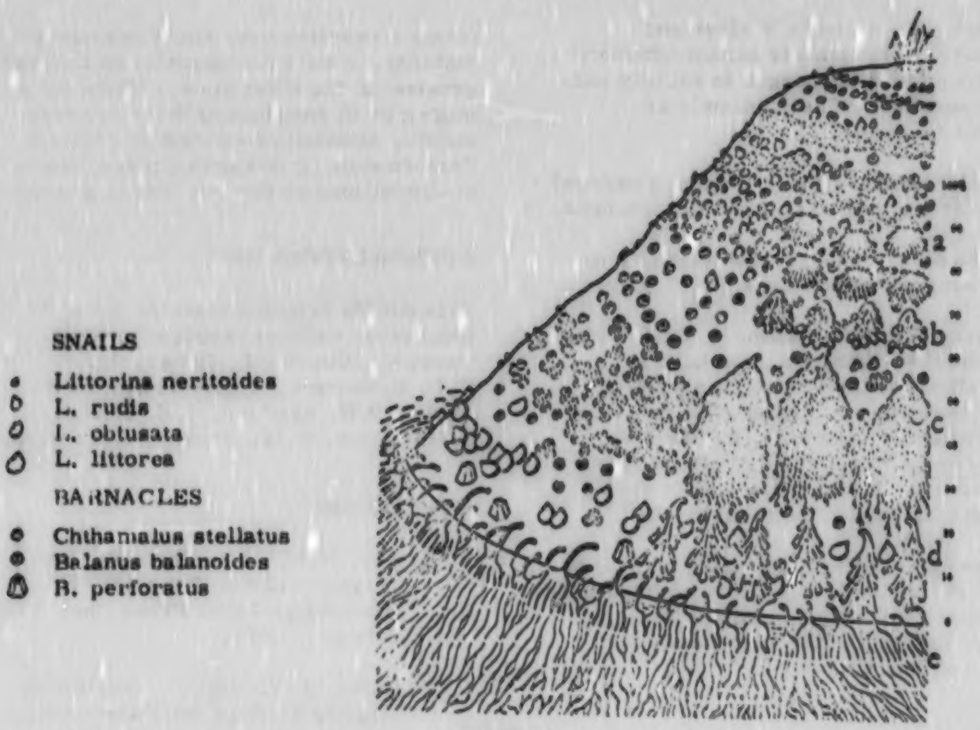

SNAILS
● Littorina neritoides
D L. rudis
O L. obtusata
O L. littorea
BARNACLES
○ Chthamalus stellatus
◉ Balanus balanoides
Δ B. perforatus
1
2
b
c
d
e

Figure 5

Zonation of plants, snails, and barnacles on a rocky shore. While this diagram is based on the situation on the southwest coast of England, the general idea of zonation may be applied to any temperate rocky ocean shore, though the species will differ. The gray zone consists largely of lichens. At the left is the zonation of rocks with exposure too extreme to support algae; at the right, on a less exposed situation, the animals are mostly obscured by the algae. Figures at the right hand margin refer to the percent of time that the zone is exposed to the air, i.e., the time that the tide is out. Three major zones can be recognized: the Littorina zone (above the gray zone); the Balanoid zone (between the gray zone and the laminarias); and the Laminaria zone. a. Pelvetia canaliculata; b. Fucus spiralis; c. Ascophyllum nodosum; d. Fucus serratus; e. Laminaria digitata. (Based on Stephenson)

2 In general, animals in river and oceanic environments cannot withstand large and rapid changes in salinity and temperature. These animals are classified as:

  a Stenohaline ("steno" meaning narrow) - narrow tolerance to salinity changes.

  b Stenothermal - narrow tolerance to temperature changes.

3 Among euryhaline animals, those living in lowered salinities often have a smaller maximum size than those of the same species living in more saline waters. For example, the lamprey (Petromyzon marinus) attains a length of 30 - 36" in the sea, while in the Great Lakes the length is 18 - 24".

4 Usually the larvae of marine organisms are more sensitive to changes in salinity than are the adults. This characteristic limits both the distribution and size of populations.

B Tides

Tidal fluctuation is a phenomenon unique to the seas (with minor exceptions). It is a twice daily rise and fall in the sea level caused by the complicated interaction of many factors including sun, moon, and the daily rotation of the earth. Tidal heights vary from day to day and place to place, and are often accentuated by local meteorological conditions. The rise and fall may range from a few inches or less to fifty feet or more.

V FACTORS AFFECTING THE PRODUCTIVITY OF THE MARINE ENVIRONMENT

The sea is in continuous circulation. Without circulation, nutrients of the ocean would eventually become a part of the bottom and biomass production would cease. Generally, in all oceans there exists a warm surface layer which overlies the colder water and forms a two-layer system of persistent stability. Nutrient concentration is usually greatest in the lower zone. Wherever a mixing or disturbance of these two layers occurs, biomass production is greatest. Factors causing this breakup are, therefore, of utmost importance concerning productivity.

ACKNOWLEDGEMENT:

This outline contains selected material from other outlines prepared by C.M. Tarzwell, Charles L. Brown, Jr., C.G. Gunnerson, W. Lee Trent, W.B. Cooke, B.H. Ketchum, J.K. McNulty, J.L. Taylor, R.M. Sinclair, and others.

REFERENCES

1 Harvey, H.W. The Chemistry and Fertility of Sea Water (2nd Ed.). Cambridge Univ. Press, New York. 234 pp. 1957.

2 Hedgpeth, J.W. (Ed.). Treatise on Marine Ecology and Paleoecology. Vol. I. Ecology Mem. 67 Geol. Soc. Amer., New York. 1296 pp. 1957.

3 Hill, M.N. (Ed.). The Sea. Vol. II. The Composition of Sea Water Comparative and Descriptive Oceanography. Interscience Publs. John Wiley & Sons, New York. 554 pp. 1963.

4 Moore, H.B. Marine Ecology. John Wiley & Sons, Inc., New York. 493 pp. 1958.

5 Reid, G.K. Ecology of Inland Waters and Estuaries. Reinhold Publ. Corp. New York. 375 pp. 1961.

6 Sverdrup, Johnson, and Fleming. The Oceans. Prentice-Hall, Inc., New York. 1087 pp. 1942.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45268.

THE AQUATIC ENVIRONMENT

Part 5: Tidal Marshes

I INTRODUCTION: The Marsh and the Estuary

A "There is no other case in nature, save in the coral reefs, where the adjustment of organic relations to physical condition is seen in such a beautiful way as the balance between the growing marshes and the tidal streams by which they are at once nourished and worn away." (Shaler, 1886)

B Estuarine pollution studies are usually devoted to the dynamics of the circulating water, its chemical, physical, and biological parameters, bottom deposits, etc.

C It is easy to overlook the intimate relationwhips which exist between the bordering marshland, the moving waters, the tidal flats, subtidal deposition, and seston whether of local, oceanic, or riverine origin.

D The tidal marsh (some inland areas also have salt marshes) is generally considered to be the marginal areas of estuaries and coasts in the intertidal zone which are dominated by emergent vegetation. They generally extend inland to the farthest point reached by the spring tides, where they merge into freshwater swamps and marshes (Figure 1). They may range in width from nonexistent on rocky coasts to many kilometers.

II MARSH ORIGINS AND STRUCTURES

A In general, marsh substrates are high in organic content, relatively low in minerals and trace elements. The upper layers bound together with living roots called turf, underlaid by more compacted peat type material.

1 Rising or eroding coastlines may expose peat from ancient marsh growth to wave action which cuts into the soft peat rapidly (Figure 2). Such banks are likely to be cliff-like, and are often undercut. Chunks of peat are often found lying about on harder substrate below high tide line. If face of cliff is well above high water, overlying vegetation is likely to be typically terrestrial of the area. Marsh type vegetation is probably absent.

2 Low lying deltaic, or sinking coastlines, or those with low energy wave action are likely to have active marsh formation in progress (Figure 3). Sand dunes are also common in such areas (Figure 4). General coastal configuration is a factor.

a Rugged or precipitous coasts or slowly rising coasts, typically exhibit narrow shelves, sea cliffs, fjords, massive beaches, and relatively less marsh area (Figure 5). An Alaskan fjord subject to recent catastrophic subsidence and rapid deposition of glacial flour shows evidence of the recent encroachment of saline waters in the presence of recently buried trees and other terrestrial vegetation, exposure of layers of salt marsh peat along the edges of channels, and a poorly compacted young marsh turf developing at the new high water level (Figure 6).

b Low lying coastal plains tend to be fringed by barrier islands, broad estuaries and deltas, and broad associated marshlands (Figure 7, 14). Deep tidal channels fan out through innumerable branching and often interconnecting rivul[illegible] The intervening grassy plains are essentially at mean high tide level.

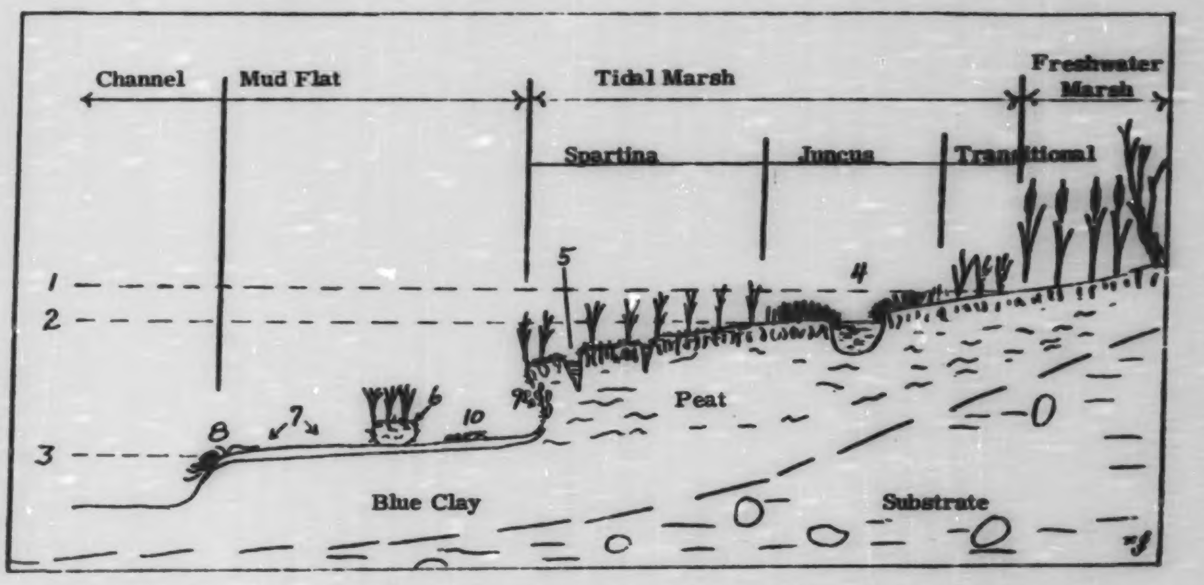

Channel
Mud Flat
Tidal Marsh
Freshwater Marsh
Spartina
Juncus
Transitional
1
2
3
4
5
6
7
8
9
10
Peat
Blue Clay
Substrate

Figure 1. Zonation in a positive New England estuary. 1. Spring tide level, 2. Mean high tide, 3. Mean low tide, 4. Bog hole, 5. Ice cleavage pool, 6. Chunk of Spartina turf deposited by ice, 7. Organic ooze with associated community, 8. eelgrass (Zostera), 9. Ribbed mussels (modiolus)-clam (mya) - mud snail (Nassa) community. 10. Sea lettuce (Ulva)

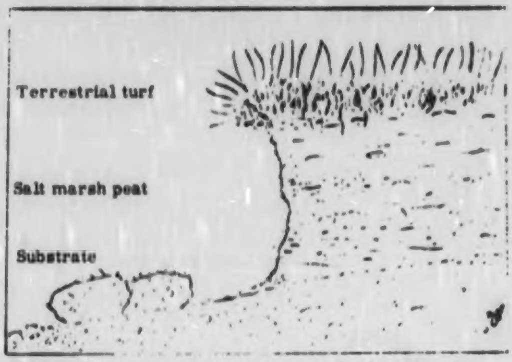


Figure 2. Diagrammatic section of eroding peat cliff

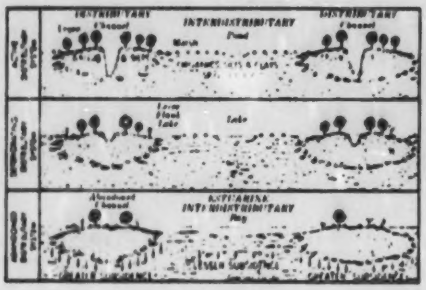


Figure 3. Effects of deltaic subsidence during distributary system abandonment

c Tropical and subtropical regions such as Florida, the Gulf Coast, and Central America, are frequented by mangrove swamps. This unique type of growth is able to establish itself in shallow water and move out into progressively deeper areas (Figure 8). The strong deeply embedded roots enable the mangrove to resist considerable wave action at times, and the tangle of roots quickly accumulates a deep layer of organic sediment. Mangroves are often considered to be effective as land builders. When fully developed, a mangrove swamp is an impenetrable thicket of roots over the tidal flat affording shelter to a sort of semi-aquatic organism such as various molluscs and crustaceans, and providing access from the nearby land to predaceous birds, reptiles and mammals.

Mangroves are not restricted to estuaries, but may develop out into shallow oceanic lagoons, or upstream into relatively fresh waters.

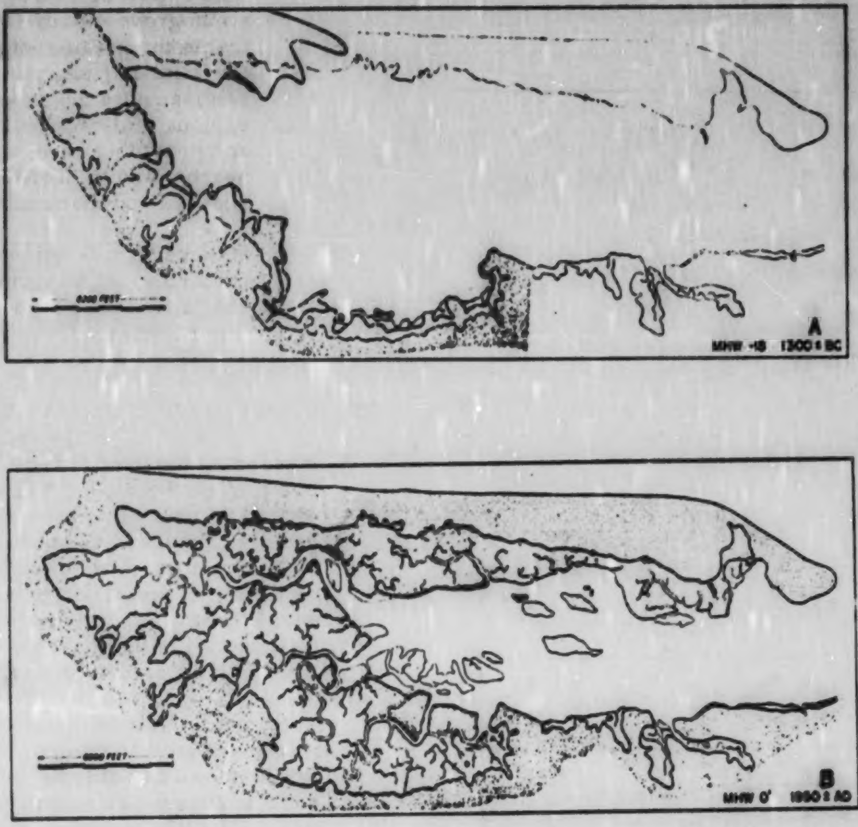
III PRODUCTIVITY OF MARSHES

A Measuring the productivity of grasslands is not easy, because grass is seldom used directly as such by man. It is thus usually expressed as production of meat, milk, or in the case of salt marshes, the total crop of animals that obtain food per unit of area. The primary producer in a tidal marsh is the marsh grass, but very little of it is used as grass. (Table 1)

The actual nutritional analysis of several marsh grasses as compared to dry land hay is shown in Table 2. A study of the yield of Juncus per square meter in a North Carolina marsh is shown in Figure 9.

B The actual utilization of marsh grass is accomplished primarily by its decomposition and ingestion by micro flora and fauna. A small quantity of seeds and solids is probably consumed directly by birds (Figure 10).

1 The quantity of micro invertebrates which thrive on this wealth of decaying marsh hay has not been estimated, nor has the actual production of small fishes such as the top minnows (Fundulus) which swarm in at high tide, or the mud snails (Nassa) and others. Many forms of oceanic life migrate into the estuaries, especially the marsh areas, for important portions of their life histories as has been mentioned elsewhere (Figure 11).



A
MHW -18 1300 ± BC
B
MHW 0' 1950 ± AD

Figure 4

Development of a Massachusetts Marsh since 1300 BC, involving an 18 foot rise in water level. Shaded area indicates sand dunes. Note meandering marsh tidal drainage. A: 1300 BC, B: 1950 AD.

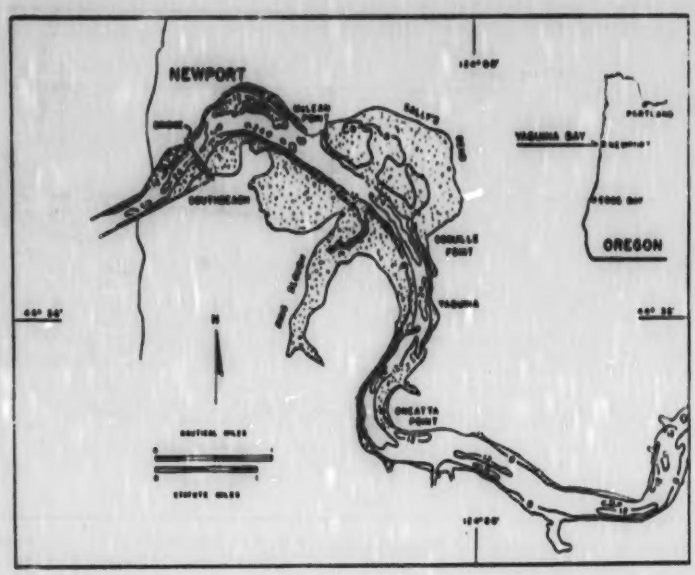


Figure 5. A River Mouth on a Slowly Rising Coast. Note absence of deltaic development and relatively little marshland, although mud flats stippled are extensive.

2 An indirect approach in Rhode Island revealed in a single August day on a relatively small marsh area, between 700 and 1000 wild birds of 12 species, ranging from 100 least sandpipers to uncountable numbers of seagulls. One food requirement estimate for three-pound poultry in the confined inactivity of a poultry yard is approximately one ounce per pound of bird per day. One-hundred (100) black bellied plovers at approximately ten (10) ounces each would weigh on the order of sixty (60) pounds. At the same rate of food consumption, this would indicate nearly four (4) pounds of food required for this species alone. The much greater activity of the wild birds would obviously greatly increase their food requirements, as would their relatively smaller size.

Considering the range of foods consumed, the sizes of the birds, and the fact that at certain seasons, thousands of migrating ducks and others pause to feed here, the enormous productivity of such a marsh can be better understood.

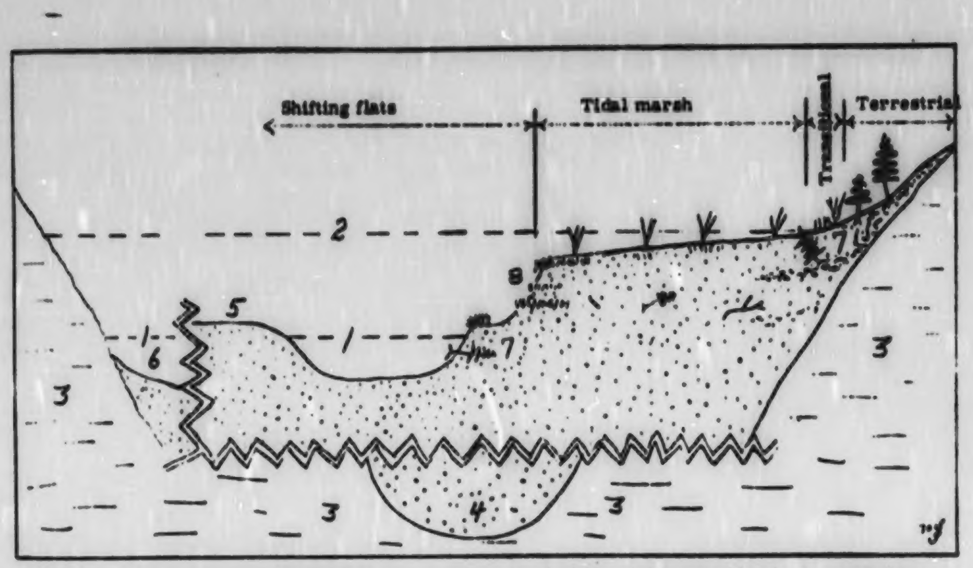

Shifting flats
Tidal marsh
Transitional
Terrestrial
1
2
3
4
5
6
7
8

Figure 6. Some general relationships in a northern fjord with a rising water level. 1. mean low water, 2. maximum high tide, 3. Bedrock, 4. Glacial flour to depths in excess of 400 meters, 5. Shifting flats and channels, 6. Channel against bedrock, 7. Buried terrestrial vegetation, 8. Outcroppings of salt marsh peat.

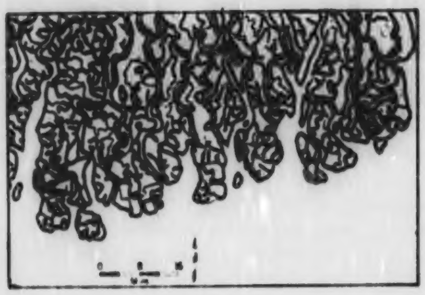


Figure 7. A Coastal Plain Marsh in India subject to a high tidal range.

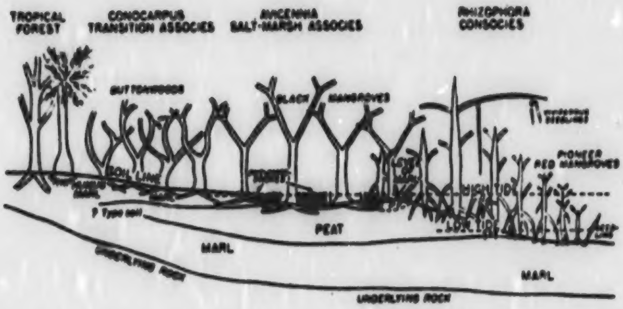

TROPICAL FOREST
CONOCARPUS TRANSITION ASSOCIES
AVICENNIA SALT-MARSH ASSOCIES
RHIZOPHORA CONSOCIES
BUTTONWOODS
BLACK MANGROVES
PIONEER RED MANGROVES
SOIL LINE
HIGH TIDE
LOW TIDE
PEAT
MARL
MARL
UNDERLYING ROCK
UNDERLYING ROCK

Figure 8. Diagrammatic transect of a mangrove swamp showing transition from marine to terrestrial habitat.

TABLE 1. General Orders of Magnitude of Gross Primary Productivity in Terms of Dry Weight of Organic Matter Fixed Annually

Ecosystem	gms/M^2/year (grams/square meters/year)	lbs/acre/year
Land deserts, deep oceans	Tens	Hundreds
Grasslands, forests, eutrophic lakes, ordinary agriculture	Hundreds	Thousands
Estuaries, deltas, coral reefs, intensive agriculture (sugar cane, rice)	Thousands	Ten-thousands

TABLE 2. Analyses of Some Tidal Marsh Grasses

T/A	Percentage Composition					
Dry Wt.	Protein	Fat	Fiber	Water	Ash	N-free Extract
Distichlis spicata (pure stand, dry)						
2.8	5.3	1.7	32.4	8.2	6.7	45.5
Short *Spartina alterniflora* and *Salicornia europaea* (in standing water)						
1.2	7.7	2.5	31.1	8.8	12.0	37.7
Spartina alterniflora (tall, pure stand in standing water)						
3.5	7.6	2.0	29.0	8.3	15.5	37.3
Spartina patens (pure stand, dry)						
3.2	6.0	2.2	30.0	8.1	9.0	44.5
Spartina alterniflora and *Spartina patens* (mixed stand, wet)						
3.4	6.8	1.9	29.8	8.1	10.4	42.8
Spartina alterniflora (short, wet)						
2.2	8.8	2.4	30.4	8.7	13.3	36.3
Comparable Analyses for Hay						
1st cut	6.0	2.0	36.2	6.7	4.2	44.9
2nd cut	13.0	3.7	28.5	10.4	5.9	38.5

Analyses performed by Roland W. Gilbert, Department of Agricultural Chemistry, U. R. I.

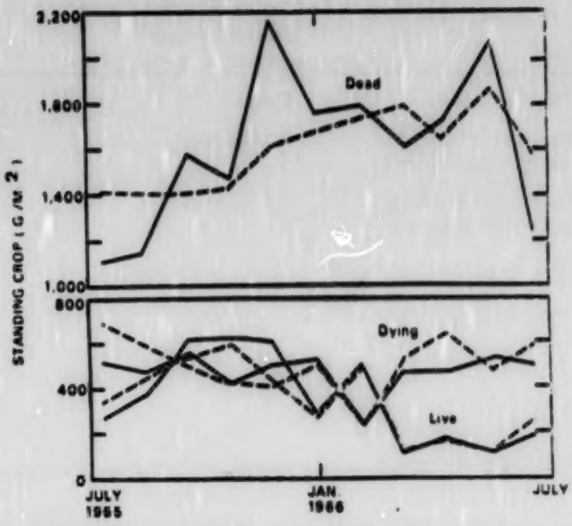


Figure 9. Standing crop of Juncus. Solid line represents observed values; broken line represents seasonal cycle calculated on the basis of an assumed constant total biomass.

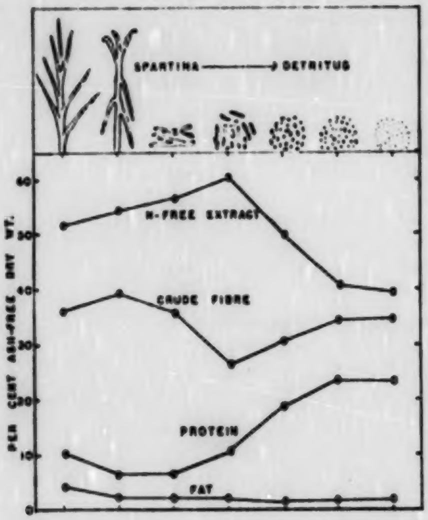


Figure 10. The nutritive composition of successive stages of decomposition of Spartina marsh grass, showing increase in protein and decrease in carbohydrate with increasing age and decreasing size of detritus particles.

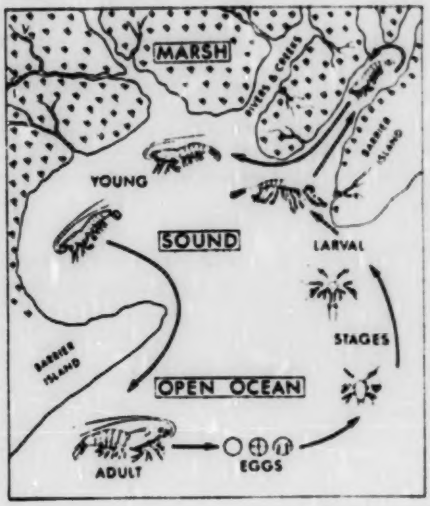

MARSH
RIVERS & CREEKS
BARRIER ISLAND
YOUNG
SOUND
LARVAL
STAGES
BARRIER ISLAND
OPEN OCEAN
ADULT
EGGS

Figure 11. Diagram of the life cycle of white shrimp (after Anderson and Lunz 1965).

A-2-36

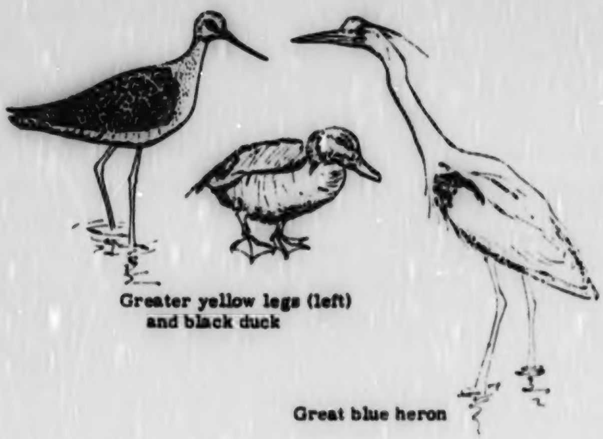

Greater yellow legs (left) and black duck
Great blue heron

Figure 12. Some Common Marsh Birds

REFERENCES

1 Anderson, W.W. The Shrimp and the Shrimp Fishery of the Southern United States. USDI, FWS, BCF. Fishery Leaflet 589. 1966.

2 Dewey, E.S., Jr. Bogs. Sci. Am. Vol. 100 (4):115-122. October 1958.

3 Emery, K.O. and Stevenson. Estuaries and Lagoons. Part II, Biological Aspects by J.W. Hedgepeth, pp. 693-728. in: Treatise on Marine Ecology and Paleoecology. Geol. Soc. Am. Mem. 67. Washington, DC. 1957.

4 Morgan, J.P. Ephemeral Estuaries of the Deltaic Environment in: Estuaries, pp. 115-120. Publ. No. 83, Am. Assoc. Adv. Sci. Washington, DC. 1967.

5 Odum, E.P. The Role of Tidal Marshes in Marine Production. The Conservationist (NY), June-July. 1961.

6 Odum, E.P. and Dela Crug, A.A. Particulate Organic Detritus in a Georgia Salt Marsh - Estuarine Ecosystem. in: Estuaries, pp. 383-388, Publ. No. 83, Am. Assoc. Adv. Sci. Washington, DC. 1967.

7 Redfield, A.C. The Ontogeny of a Salt Marsh Estuary. in: Estuaries, pp. 108-114. Publ. No. 83, Am. Assoc. Adv. Sci. Washington, DC. 1967.

8 Stuckey, O.H. Measuring the Productivity of Salt Marshes. Maritimes (Grad School of Ocean., U.R.I.) Vol. 14 (1): 9-11. February 1970.

9 Williams, R.B. Compartmental Analysis of Production and Decay of Juncus roemerianus. Prog. Report, Radiobiol. Lab., Beaufort, NC, Fiscal Year 1968, USDI, BCF, pp. 10-12.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45268.

BIOLOGICAL PARAMETERS

I INTRODUCTION

A The much used word "parameter" is defined by many dictionaries as a term with statistical implications. One definition reads: "...a quantity (or value)* that describes a statistical population".

B This definition is appropriately applied in much biological reporting.

C Objectives of this presentation are to:

1 Define and clarify the biological use of the word parameter and associated terms as used in pollution ecology.

2 To cite and examine the nature of selected biological parameters.

3 To briefly compare chemical and physical parameters with biological.

D Emphasis will be given to environmental parameters.

II SEMANTICS

A A biological parameter is a "fact of nature", a real attribute of a biological population. There may or may not be an environmental response.

B A biological parameter is measurable or estimatable. Factors or things which can be measured or estimated numerically are sometimes said to be "parametric", such as a TL_m^t, or the productivity of a reservoir.

C Non-measurable things are sometimes in the same context referred to as non-parametric; for example: the "quantity of sewage consumed by various organisms in a community" (sewage is not defined, there is no specification as to what organisms or trophic levels are implied, and so on); or "the weight of the fish held by the man in the photo".

D Some words which might be substituted for parameter in a suitable context are:

Characteristic
Factor
Feature
Attribute

III THE NATURE OF SOME BIOLOGICAL PARAMETERS

A The density of benthic invertebrates. These organisms are there, they exist. By means of certain sampling procedures we can estimate their abundance. Our estimate may be in error; different samples may vary extremely but this does not destroy the fact that the organisms are there in some finite density.

B The threshold of survival (beyond the point of no recovery) of well-fed, disease free, brook trout fingerlings 3 inches long for low DO's. Temperature 60° F, flow velocity 1 cm per second, time interval 1 hour (additional qualifications of stock, diet, water quality, and so on might be added). For the experimental population as a whole, this is a real value. Some variation between individuals would still be found.

C A TL_m^t is a parameter. It is something which is dictated by the particular combination of organisms, water, toxicants, and other conditions. It is not influenced by the method of estimation.

D Productivity of a given body of water is a parameter. The numerical value or magnitude will depend on exactly how it is defined and measured or expressed, but the fact remains that production of organic matter is taking place. The fact that our knowledge of the field is limited does not alter the fact of existence.

---

*Parenthesis by the author.

E Estimates of biological parameters may vary extremely. The fact that one bacterial test shows no coliform organisms present, and another test of the same water, shows 1,000,000 per ml does not alter the fact that at some given instant in time, the number, or standing crop of coliforms in that water has some finite value.

IV SOME FACTORS CONTRIBUTING TO VARIABILITY IN ESTIMATING BIOLOGICAL PARAMETERS

A Physical modification of certain species by pollution, e.g., knothead carp in Illinois River.

B Genetic acquisition of resistance to toxicant as mosquitofish (Gambusia) in the south.

C Collections at different times of the year would encounter different life history stages.

D Slugs of dilute solutions of certain toxicants might kill offf certain species or life history stages selectively.

E Inadequately specified, or standardized, or understood sampling techniques might provide different results under varying conditions.

F Unjustified comparisons of collections from different habitats might lead to different interpretations.

G BOD is a long-recognized parameter, its biological implications are less frequently considered.

V SOME PHYSICAL AND CHEMICAL PARAMETERS FOR COMPARISON

A DO

B pH

C Temperature

VI CONCLUSIONS

A The word parameter and appropriate derivations has a valid place in the language of pollution ecology.

B Biological parameters tend to be more variable numerically than chemical or physical parameters. Extreme values often occur. They are nonetheless equally valid and real, and often come closer to expressing a summation of environmental conditions.

REFERENCE

1 Busser, John H. On the Significance of Figures BioScience 20(13):766-767 July, 1970.

2 Gove, Philip B., ed. Websters Third New International Dictionary of the English Language. Unabridged, p. 1638. G & C Merriam Co., 1961.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, OWP, EPA, Cincinnati, OH 45268

AQUATIC ORGANISMS OF SIGNIFICANCE IN POLLUTION SURVEYS

I INTRODUCTION

A Any organism encountered in a survey is of significance. Our problem is thus not to determine which are of significance but rather to decide "what is the significance of each?"

B The first step in interpretation is recognition. "The first exercise in ecology is systematics."

C Recognition implies identification and an understanding of general relationships (systematics). The following outline will thus review the general relationships of living (as contrasted to fossil) organisms and briefly describe the various types.

II THE GENERAL RELATIONSHIPS OF LIVING ORGANISMS

A Living organisms have long been grouped into two kingdoms: plant and animal. Modern developments, however, have made this simple pattern technically untenable. It has become evident that there are as great and fundamental differences between certain other groups and these (two), as there are between the traditional "plant" and "animal." The accompanying chart consequently shows the fungi as a third "kingdom."

B The three groups are essentially defined as follows on the basis of their nutritional mechanisms (see figure 1, page 21-8.)

1 Plantae: photosynthetic; synthetizing their own organic substance from inorganic minerals. Ecologically known as PRODUCERS.

2 Animalia: ingest and digest solid particles of organic food material. Ecologically known as CONSUMERS.

3 Fungi: extracellular digestion (enzymes secreted externally.) Food material then taken in through cell membrane where it is metabolized and reduced to the mineral condition. Ecologically known as REDUCERS.

C Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms.

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal sub-groups can be defined on the basis of relative complexity of structure.

a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the higher protista.

D Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

III PLANTS

A The vascular plants are usually larger and possess roots, stems and leaves.

1 Some types emerge above the surface (emersed).

2 Submersed types typically do not extend to the surface.

3 Floating types may be rooted or free-floating.

B Algae generally smaller, more delicate, less complex in structure, possess chlorophyll like other green plants. For convenience the following artificial grouping is used in sanitary science:

1 "Blue-green algae" are typically small and lack an organized nucleus, pigments are dissolved in cell sap. Structure very simple.

2 "Pigmented flagellates" possess nuclei, chloroplasts, flagellae and a red eye spot. This is an artificial group containing several remotely related organisms, may be green, red, brown, etc.

3 "Diatoms" have "pillbox" structure of SiO_2 - may move. Extremely common. Many minute in size, but colonial forms may produce hair-like filaments. Golden brown in color.

4 "Non-motile green algae" have no locomotor structure or ability in mature condition. Another artificial group.

a Unicellular representatives may be extremely small.

b Multicellular forms may produce great floating mats of material.

IV FUNGI

Lack chlorophyll and consequently most are dependent on other organisms. They secrete extracellular enzymes and reduce complex organic material to simple compounds which they can absorb directly through the cell wall.

A Schizomycetes or bacteria are typically very small and do not have an organized nucleus.

1 Autotrophic bacteria utilize basic food materials from inorganic substrates. They may be photo-synthetic or chemosynthetic.

2 Heterotrophic bacteria are most common. They require organic material on which to feed.

B "True fungi" usually exhibit hyphae as the basis of structure.

V ANIMALS

A Lack chlorophyll and consequently feed on or consume other organisms. Typically ingest and digest their food.

B The Animal Phyla

1 PROTOZOA are single celled organisms; many resembling algae but lacking chlorophyll (cf: illustration in "Oxygen" lecture).

2 PORIFERA are the sponges; both marine and freshwater representatives.

3 CNIDARIA (= COELENTERATA) include corals, marine and freshwater jelly fishes, marine and freshwater hydroids.

4 PLATYHELMINTHES are the flat worms such as tape worms, flukes and Planaria.

5 NEMATHELMINTHES are the round worms and include both free-living forms and many dangerous parasites.

6 ROTIFERS are multicellular microscopic predators.

7 BRYOZOA are small colonial sessile forms, marine or freshwater.

8 MOLLUSCA include snails and slugs, clams, mussels and oysters, squids and octopi.

9 BRACHIOPODS are bivalved marine organisms usually observed as fossils.

10 ANNELIDS are the segmented worms such as earthworms, sludge worms and many marine species.

11 ECHINODERMS include starfish, sea urchins and brittle stars. They are exclusively marine.

12 CTENOPHORES, or comb jellies, are delicate jelly-like marine organisms.

13 ARTHROPODA, the largest of all animal phyla. They have jointed appendages and a chitinous exoskelton.

- a CRUSTACEA are divided into a cephalothorax and abdomen, and have many pairs of appendages, including paired antennae.
    - 1) CLADOCERA include Daphnia a common freshwater microcrustacean; swim by means of branched antennae.
    - 2) ANOSTRACA (=PHYLLOPODS) are the fairy shrimps, given to eruptive appearances in temporary pools.
    - 3) COPEPODES are marine and freshwater microcrustacea -- swim by means of unbranched antennae.
    - 4) OSTRACODS are like microscopic "clams with legs."
    - 5) ISOPODS are dorsoventrally compressed; called sowbugs. Terrestrial and aquatic, marine and freshwater.
    - 6) AMPHIPODA - known as scuds, laterally compressed. Marine and freshwater.
    - 7) DECAPODA - crabs, shrimp, crayfish, lobsters, etc. Marine and freshwater.
- b INSECTA - body divided into head, thorax and abdomen; 3 pairs of legs; adults typically with 2 pairs of wings and one pair of antennae. No common marine species. Nine of the twenty-odd orders include species with freshwater-inhabiting stages in their life history as follows:
    - 1) DIPTERA - two-winged flies
    - 2) COLEOPTERA - beetles
    - 3) EPHEMEROPTERA - may flies
    - 4) TRICHOPTERA - caddis flies
    - 5) PLECOPTERA - stone flies
    - 6) ODONATA - dragon flies and damsel flies
    - 7) NEUROPTERA - alder flies, Dobson flies and fish flies
    - 8) HEMIPTERA - true bugs, sucking insects such as water striders, electric light bugs and water boatman
    - 9) LEPIDOPTERA - butterflies and moths, includes a few freshwater moths
- c ARACHNIDA - body divided into cephalothorax and abdomen; 4 pairs of legs - spiders, scorpions, ticks and mites. Few aquatic representatives except for the freshwater mites and tardigrades.

C CHORDATA

1 PROTOCHORDATES - primitive marine forms such as acorn worms, sea squirts and lancelets

2 VERTEBRATES - all animals which have a backbone

- a PISCES or fishes: including such forms as sharks and rays, lampreys, and higher fishes; both marine and freshwater
- b AMPHIBIA - frogs, toads, and salamanders - marine species rare
- c REPTILA - snakes, lizards and turtles
- d MAMMALS - whales and other warm-blooded vertebrates with hair
- e AVES - birds - warm-blooded vertebrates with feathers

REFERENCE

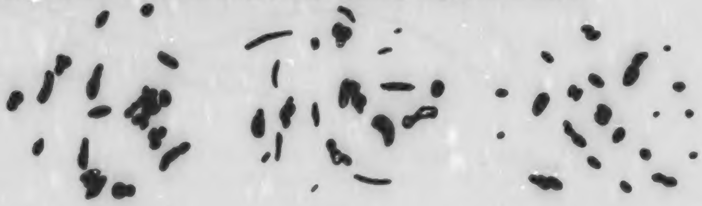
Whittaker, R.H. New Concepts of Kingdoms of Organisms. Science 163:150-160. 1969.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Quality Office, EPA, Cincinnati, OH 45226.

FUNGI

3/4

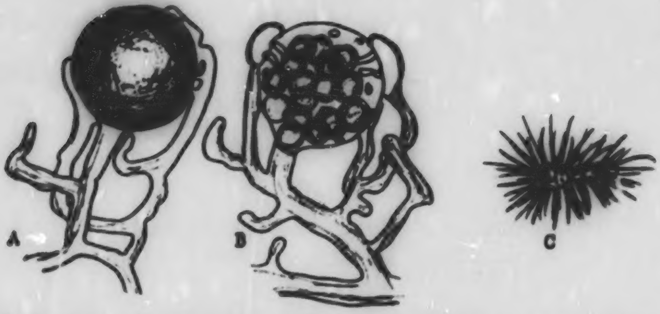
Schizomycetes - Bacteria, free living representatives



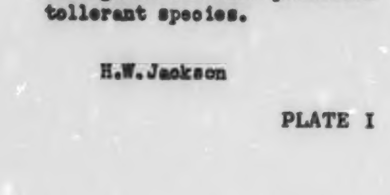
Aerobacter aerogenes

Rhizobium radicicola

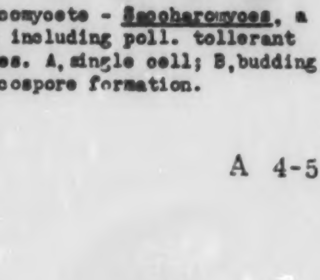
Azotobacter



Phycomycetes - *Saprolegnia*; A,detail of immature reproductive stages; B, mature oogonium and antheridia, with eggs and fertilization tubes; C, dead tadpole with growth of S.



Phycomycete - *Leptomitus*, this genus includes pollution tollerant species.



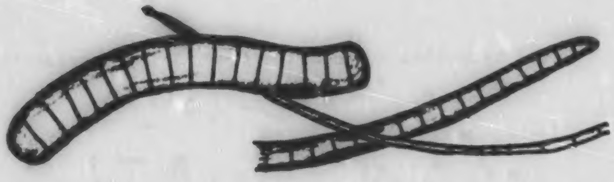
Ascomycete - *Saccharomyces*, a yeast including poll. tollerant species. A, single cell; B, budding; C, ascospore formation.

H.W.Jackson

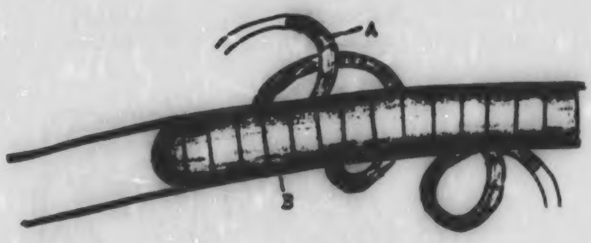
PLATE I

BLUE GREEN ALGAE

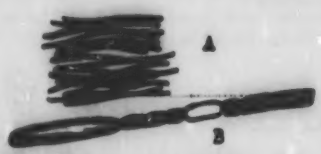
3/4



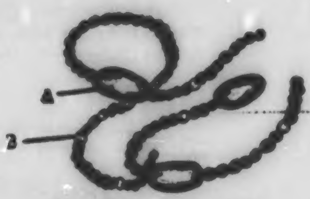
Oscillatoria spp., filaments (trichomes) range from .6 to over 60µ in diameter. Ubiquitous, pollution tollerant.



Lyngbia spp.. similar to Oscillatoria but has a sheath. A, Lyngbia contorta, reported to be generally intollerant of pollution; B, L. birgei.



Aphanizomenon flos-aquae
A, colony; B,filament



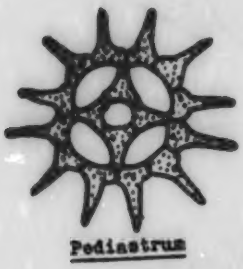
Anabaena flos-aquae
A, akinete; B,heterocyst

H.W.Jackson

PLATE II

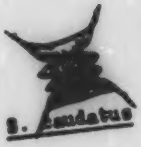
1/67

NON-MOTILE GREEN ALGAE: COCCOID
(CHLOROPHYCEAE)

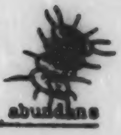


Pediastrum

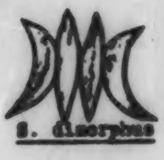
Species of the Genus Scenedesmus



S. caudatus

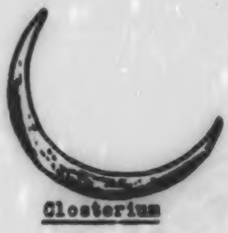


S. abundans

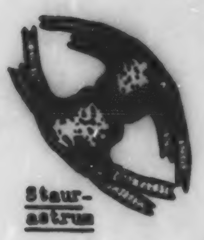


S. dimorphus

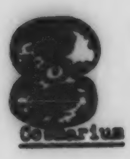
Desmids



Closterium



Staur-astrum



Cosmarium

PLATE III

11/3 NON-MOTILE GREEN ALGAE: FILAMENTOUS

(Chlorophyceae)

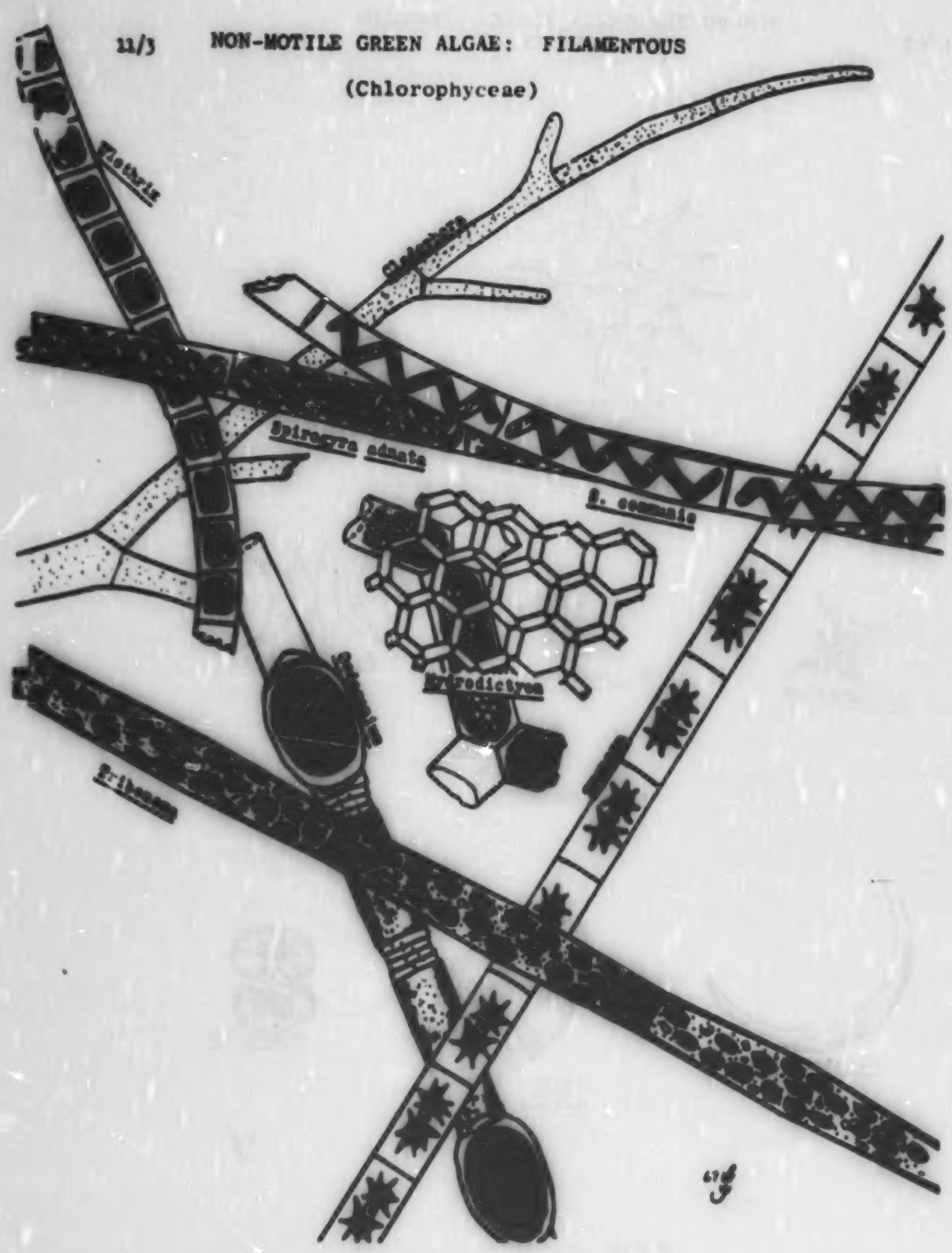
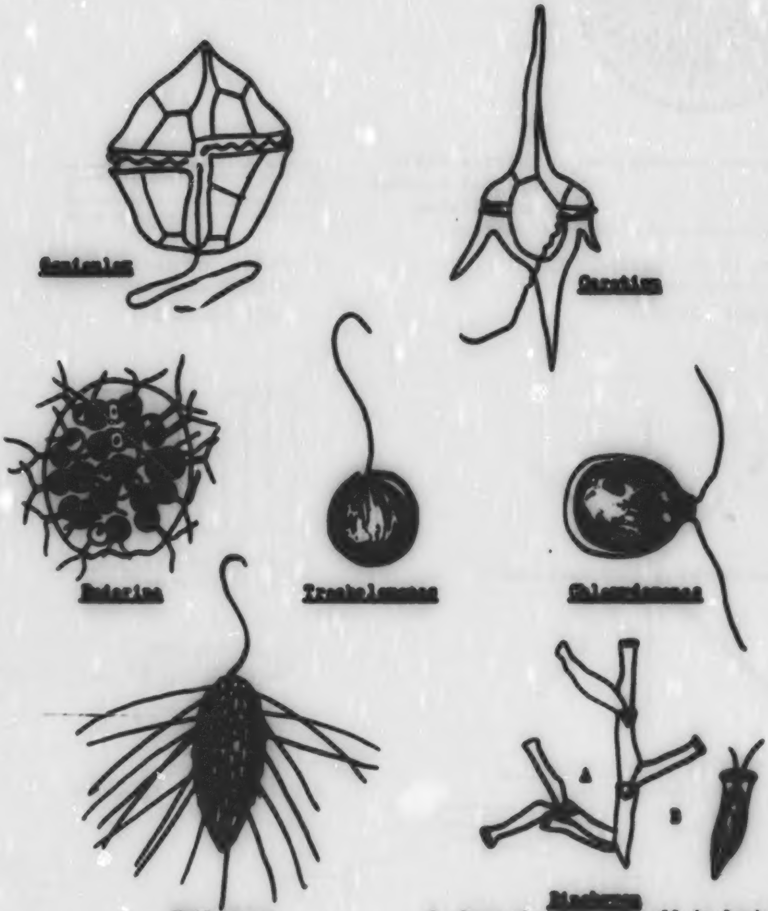

Ulothrix
Cladophora
Spirogyra adnata
S. communis
Hydrodictyon
Tribonema

PLATE IV

PIGMENTED FLAGELLATES

3/4


Gonyaulax
Ceratium
Eudorina
Trachelomonas
Chlamydomonas
Mallomonas
A
B
Dinobryon

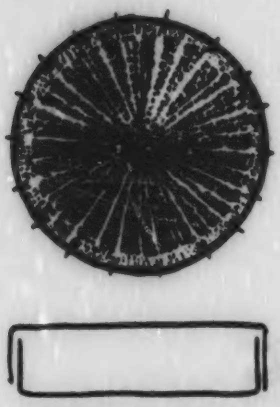
A, form of colony; B, cell in lorica.

H.W.Jackson

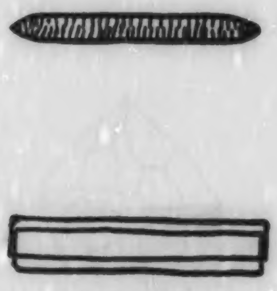
PLATE V

DIATOMS

3/4



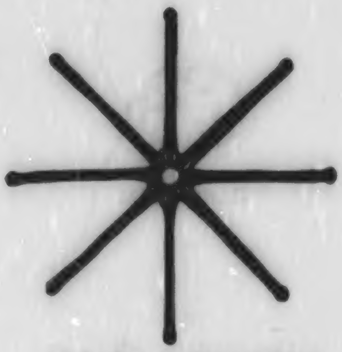
Valve views



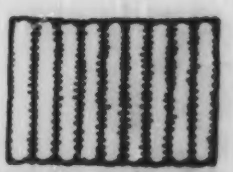
Girdle views, stylized to show basic diatom structure.

A discoid or central diatom such as *Stephanodiscus*

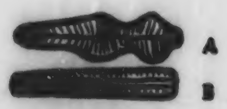
A pennate or navicular diatom such as *Fragillaria*



A colony of *Asterionella* (girdle views)



A colony of *Fragillaria* (girdle views)



Gomphonema
A,valve view; B,girdle view.

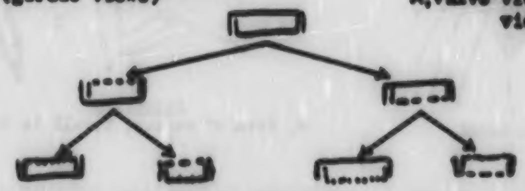


Diagram showing progressive diminution in the size of certain frustules through successive cell generations of a diatom.

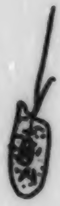
H.W.Jackson

PLATE VI

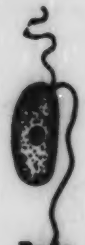
3/4

FREE LIVING PROTOZOA

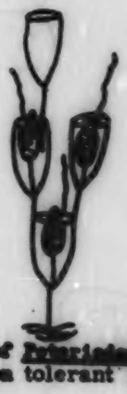
I. Flagellated Protozoa, Class Mastigophora



Anthophysis
Pollution tolerant
6 μ

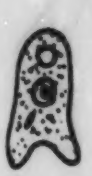


Bodo
Pollution tolerant
19 μ

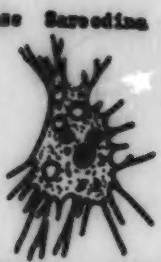


Colony of *Poteriodendron*
Pollution tolerant , 35 μ

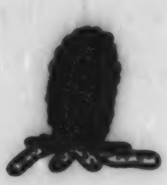
II. Ameboid Protozoa, Class Sarcodina



Dimastigamoeba
Pollution tolerant
10-50 μ

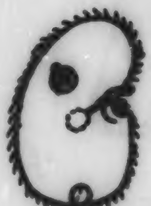


Nuclearia, reported
to be intolerant of
pollution, 45 μ.

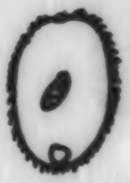


Difflugia
Pollution tolerant
60-500 μ

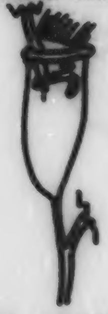
III. Ciliated Protozoa, Class Ciliophora



Colpoda
Pollution tolerant
20-120 μ



Holophrya, reported
to be intolerant of
pollution, 35 μ

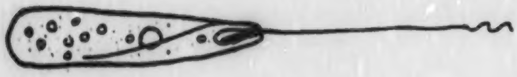


Epistylis, pollution
tolerant Colonies often
macroscopic.

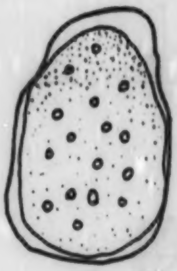
PLATE VII

H.W. Jackson

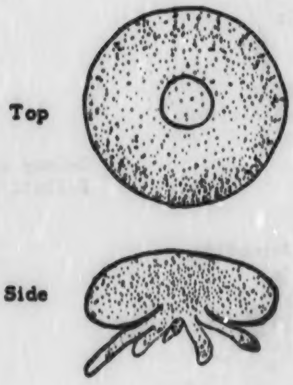
PLANKTONIC PROTOZOA



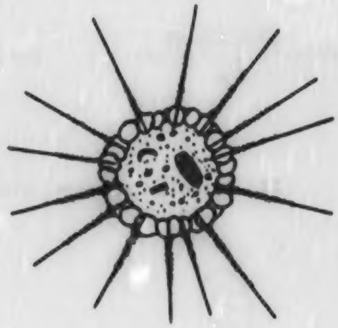
Peranema trichophorum



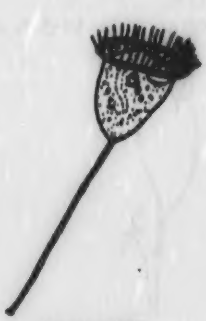
Chaos



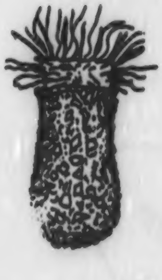
Arcella vulgaris



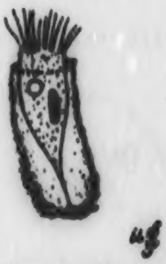
Actinosphaerium



Vorticella



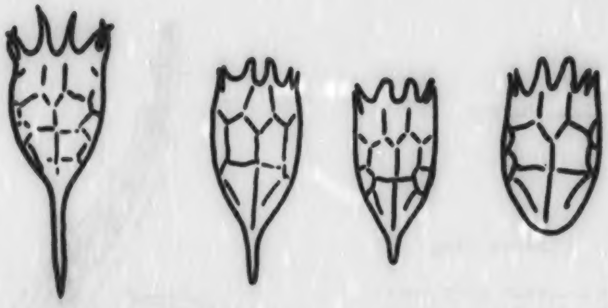
Codonella cratera



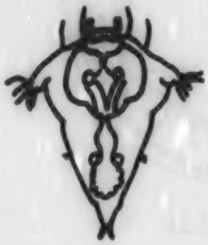
Tintinnidium fluviatile

PLATE VIII

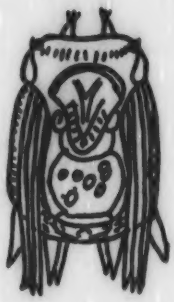
PLANKTONIC ROTIFERS



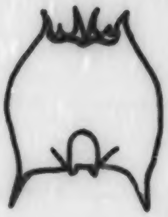
Various Forms of Keratella cochlearis



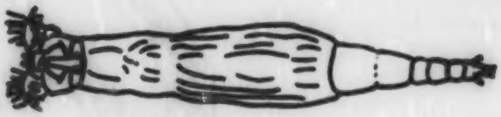
Synchaeta
pectinata



Polygarthra
vulgaris



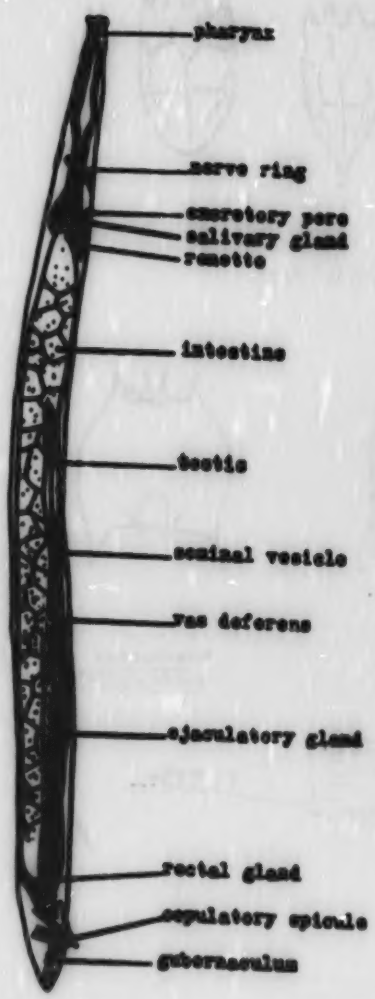
Brachionus
quadridentata



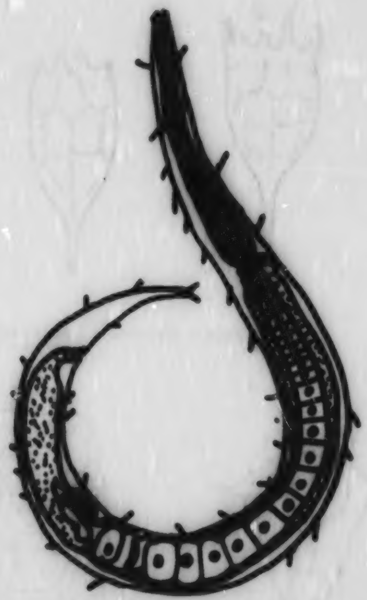
Rotaria sp

PLATE IX

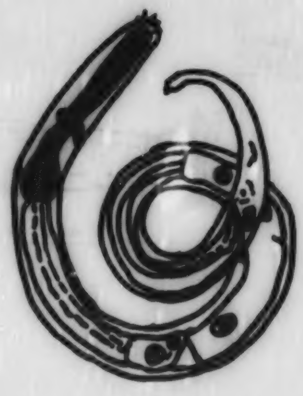
FREE LIVING NEMATHELMINTHES, OR ROUND WORMS


pharynx
nerve ring
excretory pore
salivary gland
renette
intestine
testis
seminal vesicle
vas deferens
ejaculatory gland
rectal gland
copulatory spicule
gubernaculum

Rhabditis ♂



Monhystera ♀

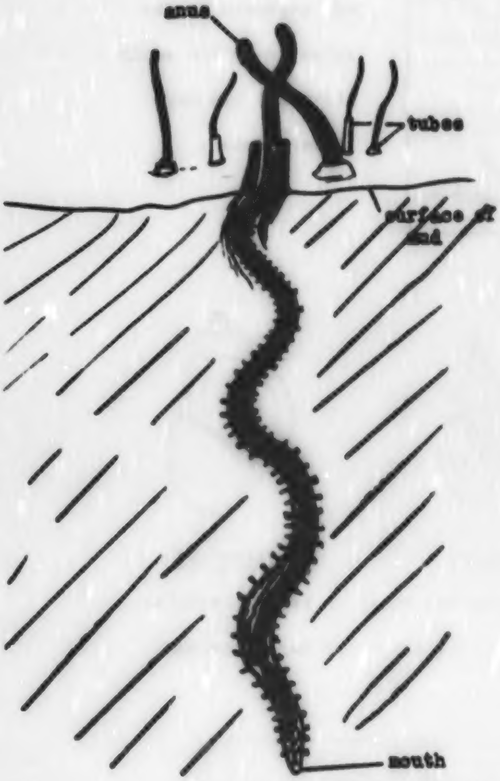


Achromadora ♀

PLATE X

FRESH WATER ANNELID WORMS

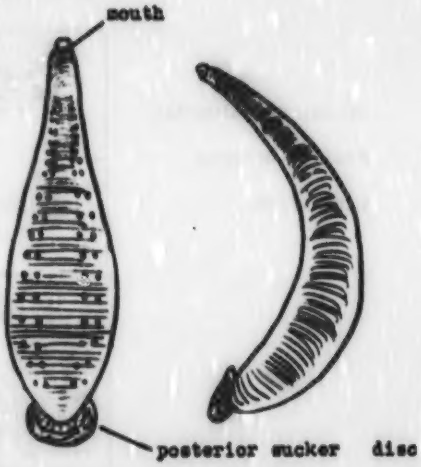
Phylum Annelida


anus
tubes
surface of mud
mouth

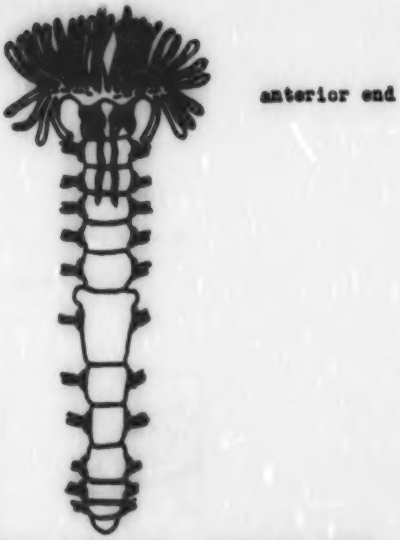
Class Oligochaeta, earthworms

Ex: Tubifex , the sludgeworm

(After Liebman)


mouth
posterior sucker disc

Class Hirudinea, leeches
(After Hegner)


anterior end

Class Polychaeta , polychaet worms

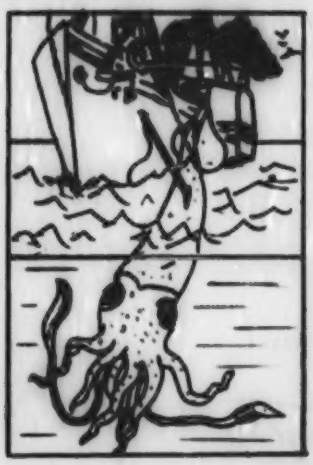
Ex: Manayunkia, a minute, rare, tube building worm. (After Leidy)

H.W.Jackson

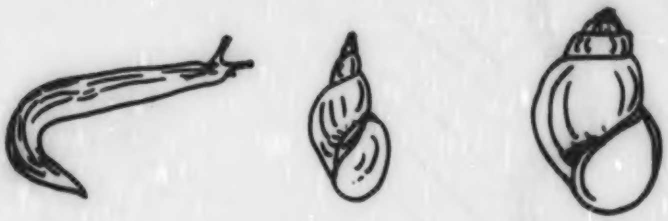
PLATE XI

SOME MOLLUSCAN TYPES

Class: Cephalopoda: Squids, octopus, cuttlefish.



Exclusively marine. The giant squid shown was captured in the Atlantic in the early nineteenth century. (After Hegner)

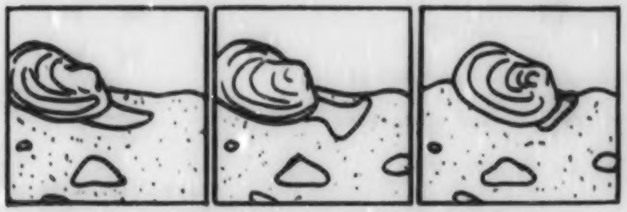


Limax, a slug

Lymnaea an air breathing snail

Campeloma a water breathing snail

Class: Gastropoda; snails and slugs. (After Buchsbaum)



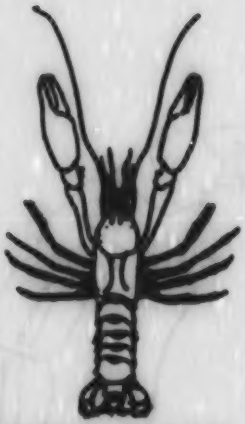
Class: Pelecypoda; clams, mussels, oysters.

Locomotion of a freshwater clam, showing how foot is extended, the tip expanded, and the animal pulled along to its own anchor. (After Buchsbaum)
H.W.Jackson

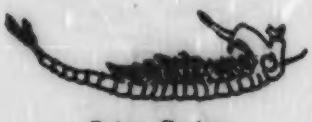
PLATE XII

Class CRUSTACEA

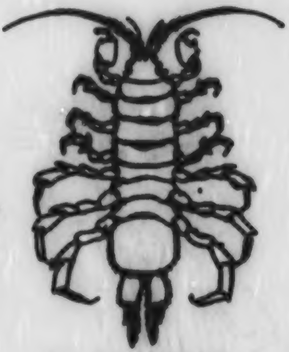
3/4



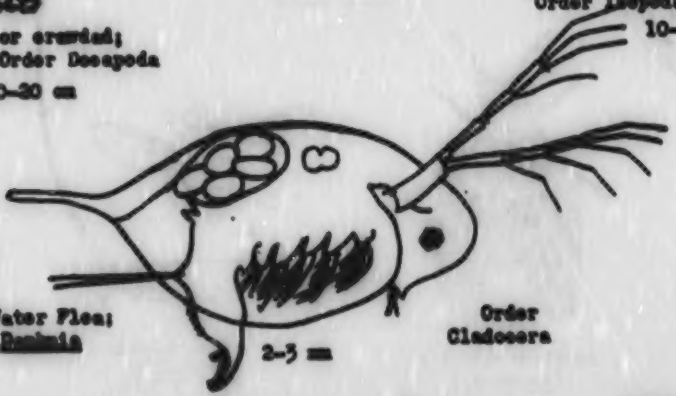
Crayfish, or crawdad;
Cambarus, Order Decapoda
10-20 cm



Fairy Shrimp;
Eubranchipus, Order
Phyllopoda
20-25 mm



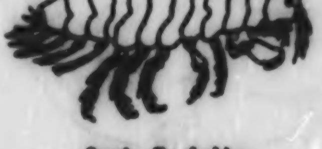
Sow Bug; *Asellus*,
Order Isopoda
10-20 mm



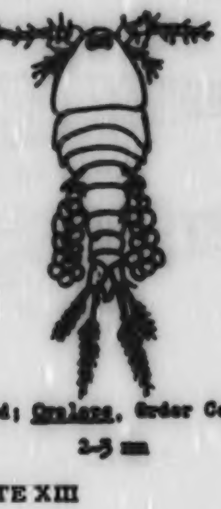
Water Flea;
Daphnia

2-3 mm

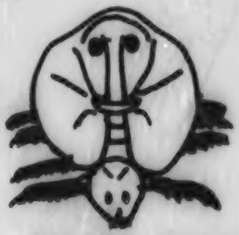
Order
Cladocera



Scud; *Hyalella*
Order Amphipoda
10-15 mm



Copepod; *Cyclops*, Order Copepoda
2-3 mm

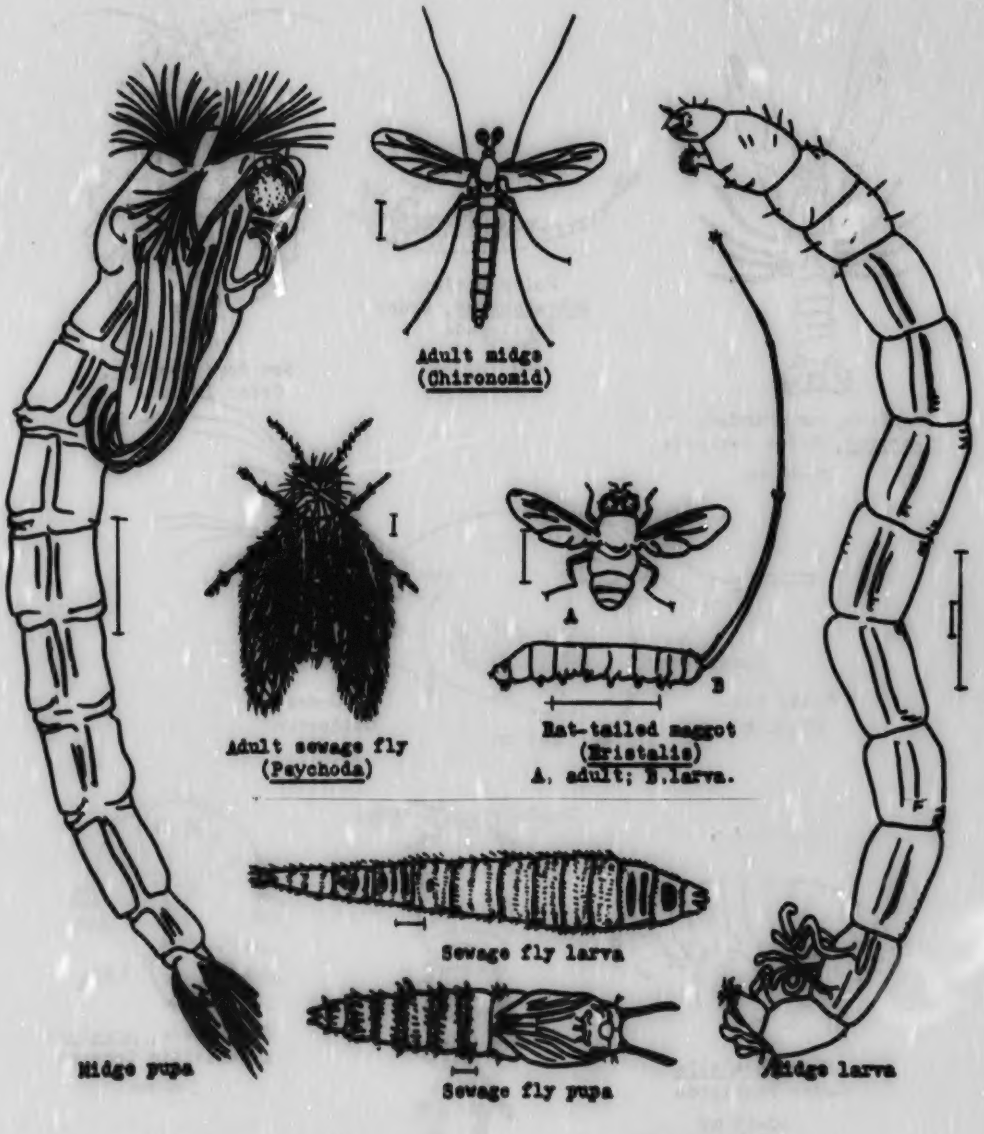


Fish Louse, *Argulus*;
a parasitic Copepod
5-6 mm

H.W.Jackson

PLATE XIII

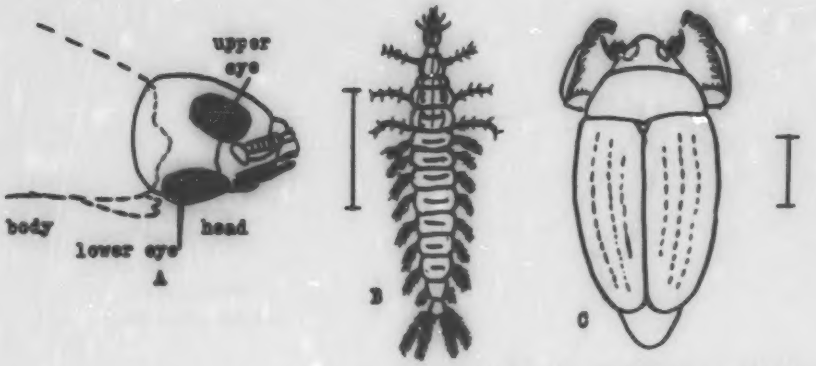
Two Winged Flies
Order DIPTERA


Adult midge
(Chironomid)
Adult sewage fly
(Psychoda)
A
B
Rat-tailed maggot
(Eristalis)
A, adult; B, larva.
Sewage fly larva
Sewage fly pupa
Midge pupa
Midge larva

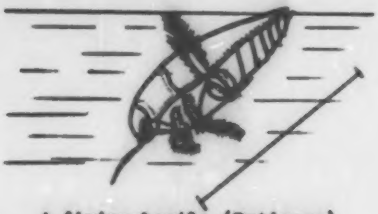
H.W.Jackson
After various authors

PLATE XIV

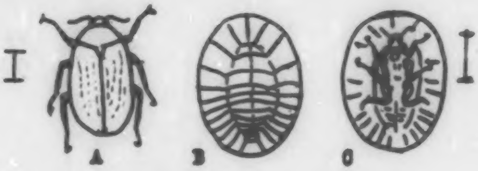
Beetles
Order COLEOPTERA



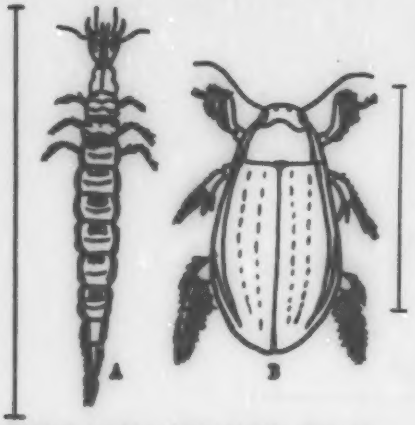
Whirligig beetle (Gyrinus) A. Side view of head of adult showing divided eye; B. Larva; C. Adult. Carnivorous.



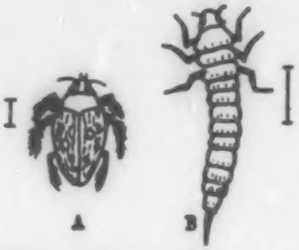
A diving beetle (Dytiscus) taking air at the surface.



The riffle beetle (Psephenus); A. adult; B. dorsal side of larva; C. ventral side of larva. Predominantly herbivorous.



A diving beetle (Cybister). The diving beetles include some of the largest and most voracious of aquatic insects. A. larva; B. adult.



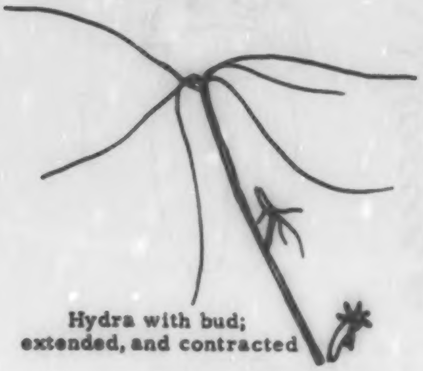
Crawling water beetle; A; adult; B. larva. Predominantly herbivorous.

H.W.Jackson. After Needham, Pennak, Morgan, and others.

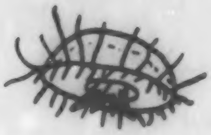
PLATE XV

MINOR PHYLA

Phylum Coelenterata

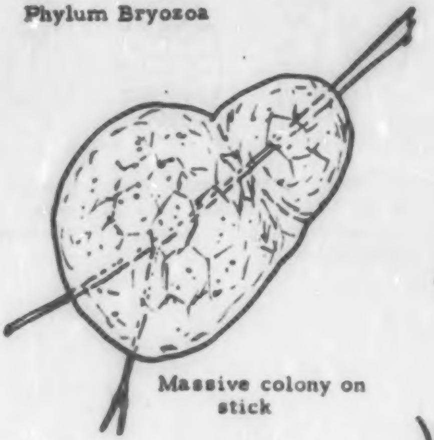


Hydra with bud;
extended, and contracted

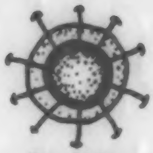


Medusa of
Craspedacusta

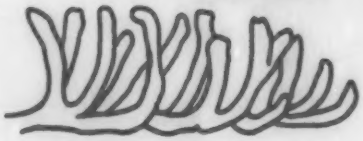
Phylum Bryozoa



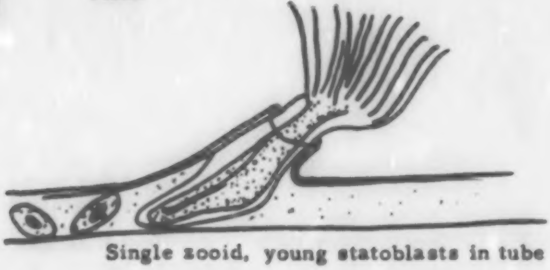
Massive colony on
stick



Statoblast



Creeping colony
on rock

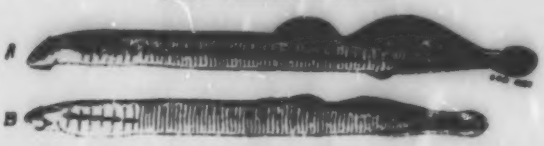


Single zooid, young statoblasts in tube

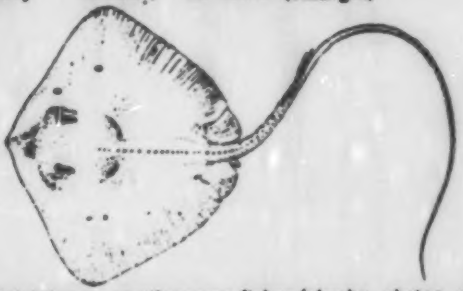
PLATE XVI

Reproduced from best available copy.

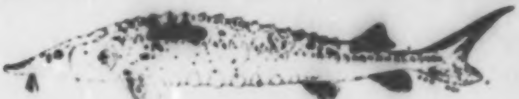
SOME PRIMITIVE FISHES



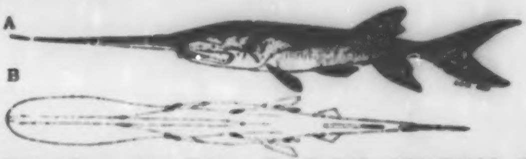
Class Agnatha, jawless fishes (lampreys and hagfishes) - Family PETROMYZONTIDAE, the lampreys. Lampetra aepyptera, the Brook Lamprey A: adult, B: larva (enlarged)



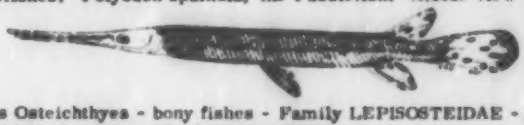
Class Chondrichthyes - cartilagenous fishes (sharks, skates, rays) Family DASYATIDAE - stingrays. Dasyatis centroura, the Roughtail Stingray



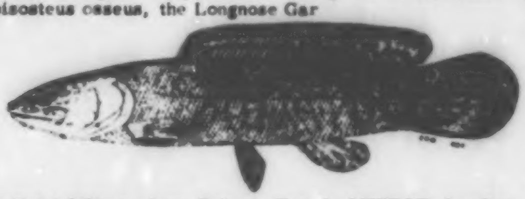
Class Osteichthyes - bony fishes - Family ACIPENSERIDAE, sturgeon. Acipenser fulvescens, the Lake Sturgeon



Class Osteichthyes - bony fishes - Family POLYODONTIDAE, the paddlefishes. Polyodon spathula, the Paddlefish. A: side view B: top view



Class Osteichthyes - bony fishes - Family LEPISOSTEIDAE - gars Lepisosteus osseus, the Longnose Gar



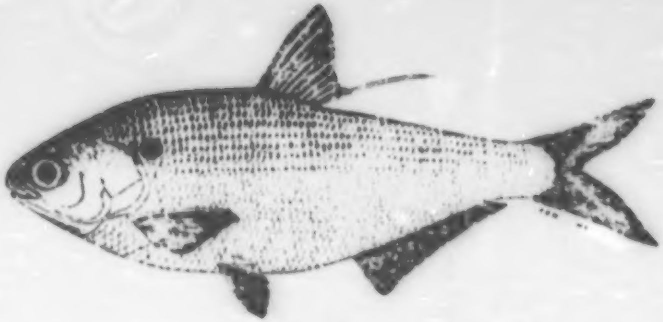
Class Osteichthyes - bony fishes - Family AMIIDAE, bowfins Amia calva, the Bowfin

Reproduced with permission; Trautman, 1957.

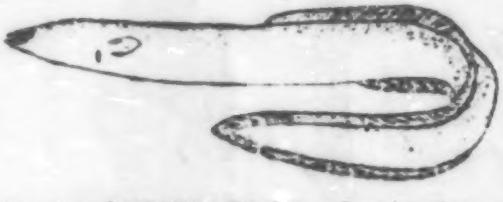
BI. AQ. pl. 91. 6. 60

PLATE XVII

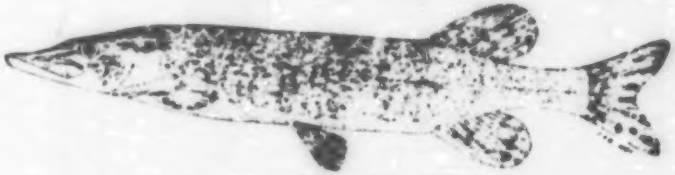
TYPES OF BONY FISHES



Family CLUPEIDAE - herrings
Dorosoma cepedianum - the eastern gizzard shad



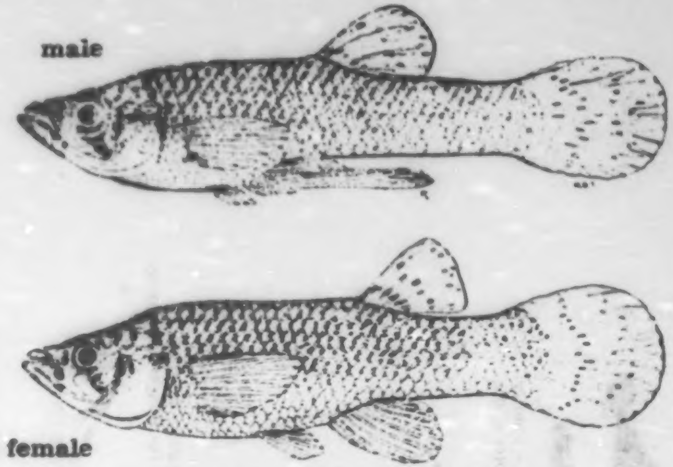
Family ANGUILLEDAE - freshwater eels
Anguilla rostrata - the American eel



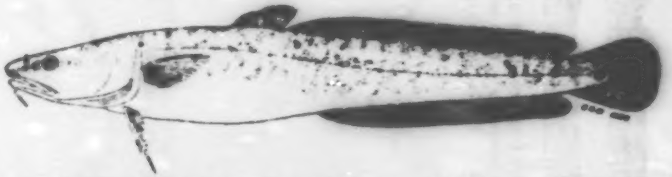
Family ESOCIDAE - pikes
Esox lucius - the northern pike

Reproduced with permission; Trautman, 1957.

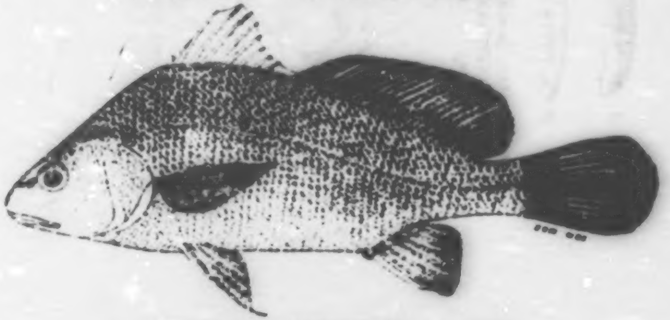
BI. AQ. pl. 9m. 6. 60



Family POECILIIDAE - livebearers
Gambusia affinis - the mosquitofish



Family GADDIDAE - codfishes, hakes, haddock, burbot
Lota lota - the eastern burbot

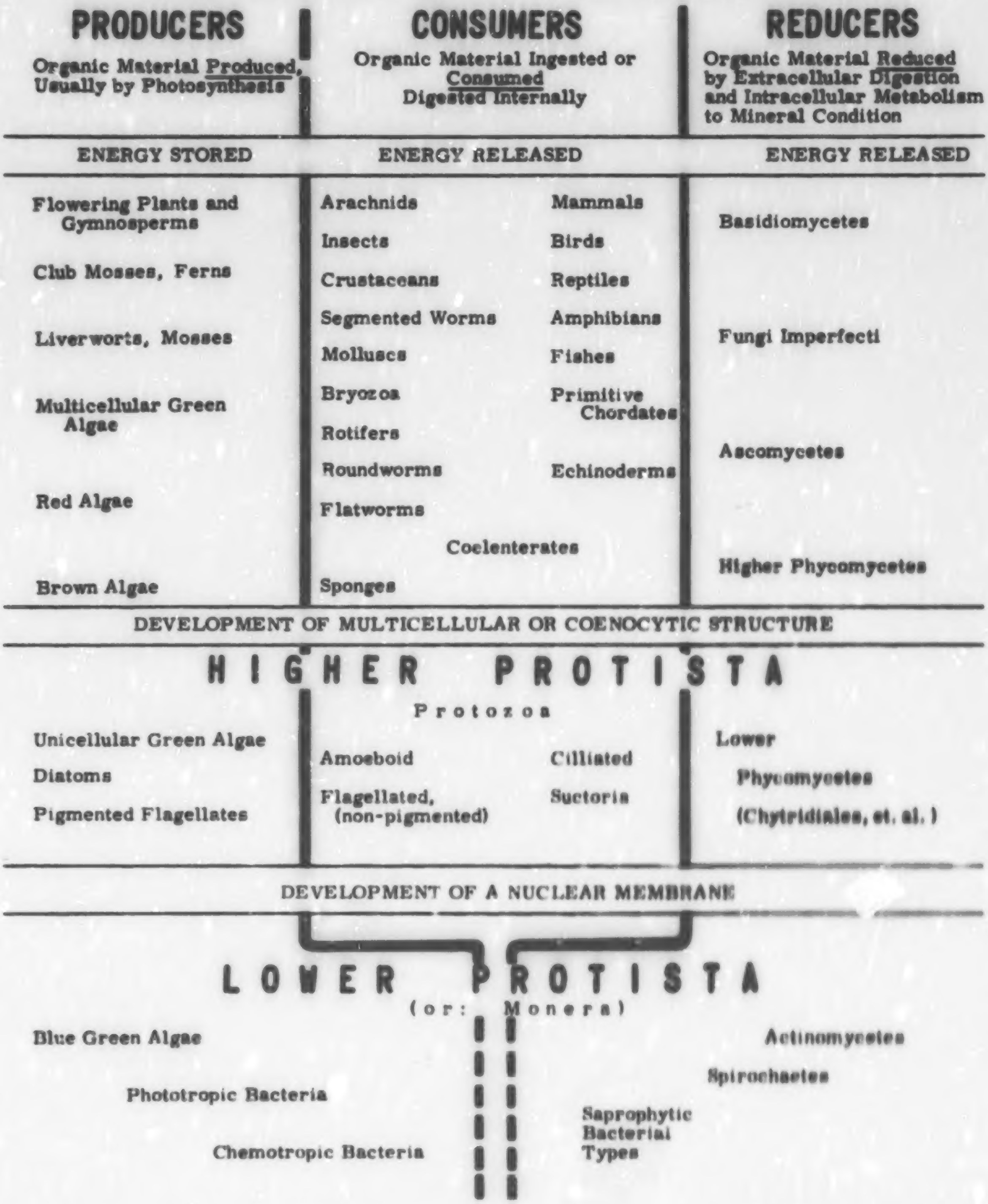


Family SCIAENIDAE - drums
Aplodinotus grunniens - the freshwater drum

PLATE XVIII

RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS

Energy Flows from Left to Right, General Evolutionary Sequence is Upward



PRODUCERS	CONSUMERS		REDUCERS
Organic Material Produced, Usually by Photosynthesis	Organic Material Ingested or Consumed Digested Internally		Organic Material Reduced by Extracellular Digestion and Intracellular Metabolism to Mineral Condition
ENERGY STORED	ENERGY RELEASED		ENERGY RELEASED
Flowering Plants and Gymnosperms	Arachnids	Mammals	Basidiomycetes
	Insects	Birds	
Club Mosses, Ferns	Crustaceans	Reptiles	
	Segmented Worms	Amphibians	
Liverworts, Mosses	Molluscs	Fishes	Fungi Imperfecti
	Bryozoa	Primitive Chordates	
Multicellular Green Algae	Rotifers		
	Roundworms	Echinoderms	Ascomycetes
Red Algae	Flatworms		
	Coelenterates		Higher Phycomycetes
Brown Algae	Sponges		

DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC STRUCTURE

HIGHER PROTISTA

	Protozoa		
Unicellular Green Algae	Amoeboid	Ciliated	Lower Phycomycetes (Chytridiales, et. al.)
Diatoms	Flagellated, (non-pigmented)	Suctoria	
Pigmented Flagellates			

DEVELOPMENT OF A NUCLEAR MEMBRANE

LOWER PROTISTA
(or: Monera)

Blue Green Algae

Phototropic Bacteria

Chemotropic Bacteria

Actinomycetes

Spirochaetes

Saprophytic Bacterial Types

GLOBAL DETERIORATION AND OUR ENVIRONMENTAL CRISIS

I FROM LOCAL TO REGIONAL TO GLOBAL PROBLEMS

A Environmental problems do not stop at national frontiers, or ideological barriers. Pollution in the atmosphere and oceans taints all nations, even those benignly favored by geography, climate, or natural resources.

1 The smokestacks of one country can pollute the air and water of another.

2 Toxic effluents poured into an international river can kill fish in a neighboring nation and ultimately pollute international seas.

B In Antarctica, thousands of miles from pollution sources, penguins and fish contain DDT in their fat. Recent layers of snow and ice on the white continent contain measurable amounts of lead. The increase can be correlated with the earliest days of lead smelting and combustion of leaded gasolines.

C International cooperation, therefore, is necessary on many environmental fronts.

1 Sudden accidents that chaotically damage the environment - such as oil spills from a tanker at sea - require international cooperation both for prevention and for cleanup.

2 Environmental effects cannot be effectively treated by unilateral action.

3 The ocean can no longer be considered a dump.

D "One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise." Aldo Leopold

II CHANGES IN ECOSYSTEMS ARE OCCURRING CONTINUOUSLY.

A Myriad interactions take place at every moment of the day as plants and animals respond to variations in their surroundings and to each other. Evolution has produced for each species, including man, a genetic composition that limits how far that species can go in adjusting to sudden changes in its surroundings. But within these limits the several thousand species in an ecosystem, or for that matter, the millions in the biosphere, continuously adjust to outside stimuli. Since interactions are so numerous, they form long chains of reactions.

B Small changes in one part of an ecosystem are likely to be felt and compensated for eventually throughout the system. Dramatic examples of change can be seen where man has altered the course of nature. It is vividly evident in his well-intentioned but poorly thought out tampering with river, lake, and other ecosystems.

1 The Aswan High Dam

2 The St. Lawrence Seaway

3 Lake Kariba

4 The Great Lakes

5 Valley of Mexico

6 California earthquake (Scientific American 3981, p. 333)

7 Everglades and the Miami, Florida Jetport

8 Copperhill, Tennessee (Copper Basin)

9 (You may add others)

C Ecosystem Stability

1 The stability of a particular ecosystem depends on its diversity. The more interdependencies in an ecosystem, the greater the chances that it will be able to compensate for changes imposed upon it.

2 A cornfield or lawn has little natural stability. If they are not constantly and carefully cultivated, they will not remain cornfields or lawns but will soon be overgrown with a wide variety of hardier plants constituting a more stable ecosystem.

3 The chemical elements that make up living systems also depend on complex, diverse sources to prevent cyclic shortages or oversupply.

4 Similar diversity is essential for the continued functioning of the cycle by which atmospheric nitrogen is made available to allow life to exist. This cycle depends on a wide variety of organisms, including soil bacteria and fungi, which are often destroyed by pesticides in the soil.

D Biological Pollution

Contamination of living native biotas by introduction of exotic life forms has been called biological pollution by Lachner et al. Some of these introductions are compared to contamination as severe as a dangerous chemical release. They also threaten to replace known wildlife resources with species of little or unknown value.

1 Tropical areas have especially been vulnerable. Florida is referred to as "a biological cesspool of introduced life".

2 Invertebrates

a Asian Clams have a pelagic veliger larvae, thus, a variety of hydro installations are vulnerable to subsequent pipe clogging by the adult clams.

b Melanian snails are intermediate hosts for various trematodes parasitic on man.

3 Vertebrates

a At least 25 exotic species of fish have been established in North America.

b Birds, including starlings and cattle egrets.

c Mammals, including nutria.

4 Aquatic plants

Over twenty common exotic species are growing wild in the United States. The problem of waterway clogging has been especially severe in parts of the Southeast.

5 Pathogens and Pests

Introduction of insect pests and tree pathogens have had severe economic effects.

III LAWS OF ECOLOGY

A Four principles have been enunciated by Dr. Barry Commoner.

1 Everything is connected to everything else.

2 Everything must go somewhere.

3 Nature knows best.

4 There is no such thing as a free lunch.

B These may be summarized by the principle, "you can't do just one thing."

IV THE THREE PRINCIPLES OF ENVIRONMENTAL CONTROL (Wolman)

A You can't escape.

B You have to organize.

C You have to pay.

V POLLUTION COMES IN MANY PACKAGES

A The sources of air, water, and land pollution are interrelated and often interchangeable.

1 A single source may pollute the air with smoke and chemicals, the land with solid wastes, and a river or lake with chemical and other wastes.

2 Control of air pollution may produce more solid wastes, which then pollute the land or water.

3 Control of wastewater effluent may convert it into solid wastes, which must be disposed of on land, or by combustion to the air.

4 Some pollutants - chemicals, radiation, pesticides - appear in all media.

B "Disposal" is as important and as costly as purification.

VI PERSISTENT CHEMICALS IN THE ENVIRONMENT

Increasingly complex manufacturing processes, coupled with rising industrialization, create greater amounts of exotic wastes potentially toxic to humans and aquatic life.

They may also be teratogenic (toxicants responsible for changes in the embryo with resulting birth defects, ex., thalidomide), mutagenic (insults which produce mutations, ex., radiation), or carcinogenic (insults which induce cancer, ex., benzopyrenes) in effect.

A Metals - current levels of cadmium, lead, and other substances whose effects on humans and fish and wildlife are not fully understood constitute a mounting concern. Mercury pollution, for example, has become a serious national problem, yet mercury has been present on earth since time immemorial. More research is needed, yet we dare not relax our standards until definitive answers have been provided.

B Pesticides

1 A pesticide and its metabolites may move through an ecosystem in many ways. Hard (pesticides which are persistent, having a long half-life in the environment includes the organochlorines, ex., DDT) pesticides ingested or otherwise borne by the target species will stay in the environment, possibly to be recycled or concentrated further through the natural action of food chains if the species is eaten. Most of the volume of pesticides do not reach their target at all.

2 Biological magnification

Initially, low levels of persistent pesticides in air, soil, and water may be concentrated at every step up the food chain. Minute aquatic organisms and scavengers, which screen water and bottom mud having pesticide levels of a few parts per billion, can accumulate levels measured in parts per million - a thousandfold increase. The sediments including fecal deposits are continuously recycled by the bottom animals.

a Oysters, for instance, will concentrate DDT 70, 000 times higher in their tissues than it's concentration in surrounding water. They can also partially cleanse themselves in water free of DDT.

b Fish feeding on lower organisms build up concentrations in their visceral fat which may reach several thousand parts per million and levels in their edible flesh of hundreds of parts per million.

c Larger animals, such as fish-eating gulls and other birds, can further concentrate the chemicals. A survey on organochlorine residues in aquatic birds in the Canadian prairie provinces showed that California and ring-billed gulls were among the most contaminated. Since gulls breed in colonies, breeding population changes can be detected and related to levels of chemical contamination. Ecological research on colonial birds to monitor the effects of chemical pollution on the environment is useful.

C "Polychlorinated biphenyls" (PCB's). PCB's are used in plasticizers, asphalt, ink, paper, and a host of other products. Action has been taken to curtail their release to the environment, since their effects are similar to hard pesticides.

D Other compounds which are toxic and accumulate in the ecosystem:

1 Phalate esters - may interfere with pesticide analyses

2 Benzophyrenes

3

VII EXAMPLES OF SOME EARLY WARNING SIGNALS THAT HAVE BEEN DETECTED BUT FORGOTTEN, OR IGNORED.

A Magnetic micro-spherules in lake sediments now used to detect changes in industrialization indicate our slowness to recognize indicators of environmental change.

B Salmonid fish kills in poorly buffered clean lakes in Sweden. Over the past years there had been a successive increase of SO_2 in the air and precipitation. Thus, air-borne contamination from industrialized European countries had a great influence on previously unpolluted waters and their life.

C Minimata, Japan and mercury pollution.

D Organochlorine levels in commercial and sport fishing stocks, ex., the lower Mississippi River fish kills.

E You may complete the following:

1

2

VIII SUMMARY

A Ecosystems of the world are linked together through biogeochemical cycles which are determined by patterns of transfer and concentrations of substances in the biosphere and surface rocks.

B Organisms determine or strongly influence chemical and physical characteristics of the atmosphere, soil, and waters.

C The inability of man to adequately predict or control his effects on the environment is indicated by his lack of knowledge concerning the net effect of atmospheric pollution on the earth's climate.

D Serious potential hazards for man which are all globally dispersed, are radionuclides, organic chemicals, pesticides, and combustion products.

E Environmental destruction is in lock-step with our population growth.

ACKNOWLEDGEMENT:

This outline has been extracted in part from the first annual report of the Council on Environmental Quality: Environmental Quality. USGPO, Washington, DC. 326 pp. $1.75. 1970.

REFERENCES

1 Goldman, Charles R. Is the Canary Dying? The time has come for man, miner of the worlds resources, to surface. Calif. Medicine 113:21-26. 1970.

2 Lachner, Ernest A., Robins, C. Richard, and Courtenay, Walter R., Jr. Exotic Fishes and Other Aquatic Organisms Introduced into North America. Smithsonian Contrib. to Zool. 59:1-29. 1970.

3 Nriagu, Jerome O. and Bowser, Carl J. The Magnetic Spherules in Sediments of Lake Mendota, Wisconsin. Water Res. 3:833-842. 1969.

4 Rhode, Wilhelm. Limnology, Social Welfare, and Lake Kinneret. Internat. Assoc. Theory and Appl. Limnology. 17:40-48. 1969.

5 Hood, Donald W. ed. Impingement of Man on the Oceans. Wiley-Interscience. 738 p. 1971.

6 Commoner, Barry. The Closing Circle, Nature, Man, and Technology. Alfred A. Knopf. 326 p. 1971.

7 Dansereau, Pierre ed. Challenge for Survival. Land, Air, and Water for Man in Megalopolis, Columbia Univ. Press. 235 p. 1970.

8 Caudill, Harry M. My Land is Dying. E. P. Dutton. 144 p. 1971.

9 Berkowitz, David A. and Squires, Arthur M. editors. Power Generation and Environmental Change. MIT Press. 441 p. 1971.

10 Wiens, John A. ed. Ecosystem Structure and Function. Oregon State Univ. Press. 176 p. 1972.

11 Burns, Noel M. and Ross, Curtis editors. Project Hypo, An Intensified Study of the Lake Erie Central Basin Hypolimnion and Related Surface Water Phenomena. 182 p. CCIW, paper no. 6 and EPA Tech. Report TS-05-71. 208-24. 1972.

12 Matthews, W. H., Smith, F. E., and Goldberg, E. D. Man's Impact on Terrestrial and Oceanic Ecosystems. MIT Press. 1971.

13 Wagner, Richard H. Environment and Man. W. W. Norton & Co., Inc. NY. 491 p. 1971.

14 Leopold, Aldo. A Sand County Almanac with Essays on Conservation from Round River. Sierra Club/Ballantine Books. 295 p. 1970.

15 Sondheimer, Ernest B. and Simeone, John B. Chemical Ecology. Academic Press. 336 p. 1970.

16 Whittaker, Robert H. Communities and Ecosystems. Macmillan Co. 162 p. 1970.

17 Environmental Quality. Second Annual Report of the Council on Environmental Quality. August 1971. Third Annual Report. August 1972.

18 Toxic Substances. Council on Environmental Quality. 25 p. April 1971.

19 Zinc in Water. A Bibliography USDI. Office Water Resources WRSIC Series 208. 1971. Also in this series WRSIC 201-207, Mercury, Magnesium, Manganese, Copper, Trace Elements, and Strontium.

This outline was prepared by Ralph M. Sinclair, Aquatic Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

SECTION B

THE NATURE AND MEANING OF POLLUTION

	Outline Number
Mechanisms of Pollution Tolerance and Sensitivity	B-1
Effects of Pollution on Aquatic Life	B-2
Nutrients: The Basis of Productivity	B-3
Biological Aspects of Natural Self Purification	B-4
The Effects of Pollution on Lakes	B-5
Significance of Eutrophication	B-6
Pollution of the Marine Environment	B-7
Water Temperature and Water Quality	B-8
Biotic Effects of Siltation	B-9
The Cycling of Radionuclides in the Aquatic Environment	B-10
Bacteria and Protozoa as Toxicological Indicators	B-11
Pathogenic Microorganisms in Water and Sewage	B-12
Effects of Pollution on Fish	B-13
Influence of Land Runoff on Water Quality	B-14
Ground Water Quality Studies	B-15
The Effects of Organisms on Pollution and the Environment	B-16
Effects and Control of Oil Pollution	B-17

MECHANISMS OF POLLUTION TOLERANCE AND SENSITIVITY

I INTRODUCTION

A The fact that some organisms are more resistant to pollution than others needs no emphasis. The matter of "why?" and "how?" on the other hand, is quite another question. In some cases the answer is obvious, in others not. In general we can say that the adaptations of certain species enable them to resist certain types of natural conditions such as organic deposits or sand bars. When man artificially creates conditions such as sludge banks, or sand bars, organisms which can tolerate such conditions move in, survive, and often thrive. Other forms are eliminated.

B The following outline will offer examples of some of the better understood mechanisms by means of which such organisms survive, and some speculation on others.

C In terms of responses, we will examine resistance vs. susceptibility. In terms of type of pollution, we will discuss organic vs. the toxic and other types of pollution.

II ORGANIC POLLUTION

A The Nature of the Problem

1 Organic pollution is essentially nontoxic.

2 Oxygen depletion, physical turbidity, and smothering blankets of sediment or sludge constitute the main hazards to aquatic life. The physical effect of sediments will be covered below.

B Devices and mechanisms for living in oxygen-poor or oxygen-free water include the following:

1 Obtaining oxygen from the air by means of periodic trips or access to the surface.

a The snorkel tube of the rat-tailed maggot.

b Periodic trips of the mosquito larvae and Corixidae or water boatmen. The mosquito takes air directly into its respiratory system, the water boatman into a trap or space beneath the wing covers, as well as into a layer of air held by fine hairs or "pile" all over its body.

c Behavior of the air breathing snails such as Physa which have an internal lung cavity.

d Insects which tap into air tubes in aquatic plants.

e Fishes which gulp air at surface or breathe surface water.

2 Special devices and behavior for respiration in water.

a Hind intestinal respiratory structures of dragonfly larvae permit respiration in silt-laden water.

b Movement of gill covers and similar structures in isopods and certain insect groups maintains a current of water over respiratory organs.

c Body movements of (Ventilation) Chironomid larvae create water current in tube. Sludge worms and other annelids also create water movement by means of sinuous body movements.

d Stoneflies do "pushups."

3 Physiological and behavorial adaptations to endure low oxygen tensions.

a Forms possessing accessory respiratory pigments such as hemoglobin might be expected to be able to extract the last vestige of dissolved oxygen from the water. Two groups famous for resisting low DO do have hemoglobin: the larvae of certain Chironomids (midge flies) and small annelid worms such as sludge worms. (It should be noted however, that the hemoglobin in each case is simply dissolved in the blood plasma, rather than being concentrated in special corpuscles as is the case in the more efficient vertebrate system.)

b The mere possession of hemoglobin however, does not seem to assure tolerance of low DO (Walshe '47). Larvae of the midge Tanytarsus spp. have hemoglobin, but will not tolerate oxygen-poor waters. Hemoglobin-bearing Chironomus bathopilus is moderately tolerant, and Chironomus plumosus is highly tolerant, however.

c During periods of low DO, Chironomus plumosus apparently respires carbohydrates as usual, but excretes excess lactic acid instead of accumulating it.

d Various species of Daphnia (micro-crustacea) have been shown to accumulate hemoglobin in oxygen-poor waters but not in oxygen-rich waters (Fox '47). However, no clear adaptive significance has yet been proven.

C Advantageous Feeding Habits

All highly pollution tolerant organisms are scavengers, and hence find an abundance of food. Most are relatively defenseless and hence have normally high reproductive rate. The results in a polluted situation is thus usually an extreme abundance.

D The reverse problem is why are intolerant species intolerant?

1 A physiological requirement for higher oxygen levels is probably most basic.

2 Turbidity would hamper any organisms employing sight in any way.

3 Absence of light would suppress the growth of green algae, and hence restrict the growth of algae feeders.

III SILT OR SEDIMENT POLLUTION

A Sediments may be biochemically degradable, thus exerting an oxygen demand. Inert silts by themselves have many damaging effects such as abrasive or smothering effects.

B Biological mechanisms for enduring inert silt or sand pollution are not numerous, and consequently such locations are usually known as biological deserts. Since some life exists even in deserts however, a few forms may occasionally be found. In general they are typical sand or mud dwellers. Since the available food in such a substrate is at best of a very low order, inhabitants of these situations must either seek buried or trapped food particles or capture food from the passing waters.

1 If there is no BOD involved, and water and oxygen circulate down into the deposit, burrowing forms such as certain mayflies, annelid worms, ammocetes lamprey larvae, micro-crustaceans, and others may burrow down to depths of two feet or more. Fish eggs normally deposited in gravel and newly hatched larvae are also dependent on circulating water. Such a population can be killed overnight by a layer of fine sediment or sludge which seals the surface to water circulation.

2 Water or plankton feeders include clams and mussels which can move about freely in a soft, shifting bottom, thus keeping on top of silt or sand as it accumulates. If deposition is actively taking place, however, there will probably be so much turbidity in the water that plankton (food) organisms are unable to live. Under such circumstances certain clams and snails have the ability to close the shell so tightly by means of valves or an operculum that all contact is lost with the environment for extended periods, during which CO_2 tends to lower the rate of body metabolism. Organisms of this type have been reported to be dug up with sand and gravel and incorporated into concrete products while still alive. Emergence of a population of asiatic clams for example, just as a big block of concrete is setting is said to be rough on contractors!

3 An interesting situation occasionally develops in estuaries where mud of moderate organic content is slowly deposited over oyster beds. The oysters are unable to move, but as they grow, their shells tend to bend upward above the accumulating silt, and may grow to several inches in length while growing very little in width. Crowding brings about a similar reaction in the effort to avoid being buried.

IV TOXIC POLLUTION

A Few generalizations can be offered relative to toxic pollution except that toxicity is relative, and all forms do not respond equally to a given toxicant.

B Few mechanisms of toleration can be listed, beyond the natural resistance that certain forms may have for a given condition. For example, some marine species may survive a salt concentration that is toxic to freshwater species.

Over a period of several generations, some species may develop a genetic resistance to some toxicant such as insecticides in the same way that DDT-resistant strains of houseflies have developed. Copper sulfate, and chlorine-resistant strains of algae such as Cosmarium for example, may develop in treated water supply reservoirs.

C Some bottom in-fauna organisms such as annelid worms may retreat down into burrows until a slug of undesirable water passes.

D Molluscs may close shells tightly for the same purposes. The metabolic rate is known to diminish with the increase of CO_2 inside the closed shell, thereby enabling them to remain tightly sealed for extended periods of time.

V EFFECTS OF LIFE HISTORY STAGES

A In order to survive in a polluted area, each life history stage of an indigenous organism must be able to survive in turn.

B In some given life history stage cannot tolerate conditions, and the species is present:

1 Fortuitous changes may occur at the proper time(s) to permit survival of the more susceptible stage(s) or:

2 Recruitment from less polluted areas may occur.

C Some examples of reproductive stages or procedures which might affect pollution sensitivity:

1 Egg or egg-like stages are often enclosed in protective membranes, jelly masses, or cases. May remain dormant until favorable conditions develop.

2 Eggs may be deposited in locations where they are less exposed to polluted water as:

buried in the gravel,
on the surface film,
on the rocks over the water moistened by spray,
on mud surface near water,
or in locations where maximum water circulation is encountered as at lip of waterfall.

3 Eggs may require minimal DO due to low metabolic rate.

4 Eggs deposited on or in bottom may be susceptible to smothering.

5 Newly hatched larvae often continue to live on stored yolk material for a time. On beginning to take natural food, they may be killed by toxic content thereof, such as insecticide.

6 Some forms such as certain sludge worms commonly reproduce by (asexual) fragmentation, hence avoiding egg and larva stages.

REFERENCES

1 Cordone, A. J., and Kelley, D. W. The Influence of Inorganic Sediment on the Aquatic Life in Streams. Calif. Fish and Game. 47:189-228. No. 2. 1961.

2 Bates, John M. The Impact of Impoundment on the Mussel Fauna of Kentucky Reservoir, Tennessee River. Amer. Midl. Nat. 68(1):232-236. 1962.

3 Fox, H. Munro. Daphnia Hemoglobin. Nature. London. P. 431. Sept. 27, 1947.

4 Walshe, Barbara M. On the Function of Hemoglobin in Chironomus after Oxygen Lack. Jour. Exp. Bol. Cambridge 124:329-342. 1947.

5 Vinson, S. B., Boyd, C. E., and Ferguson, D. E. Resistance to DDT in the Mosquito Fish Gambusia affinis Science 139:217-218. Jan. 18, 1963.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, OWP, EPA, Cincinnati, OH 45268.

EFFECTS OF POLLUTION ON AQUATIC LIFE

I INTRODUCTION

A The effluent from any given industrial plant may be combined with municipal sewage, and/or wastes from other industries. This may occur in the sewerage system or in a natural body of water.

1 Toxic wastes may inhibit biota of treatment plant as well as life in receiving stream.

2 Organic wastes may simply increase sewage-type load on plant and stream.

3 The above effects may be reinforced or neutralized by a complex of industrial wastes.

B The general overall character of a body of water may be subtly changed over a period of time.

II The principle of limiting factors (see Figure 1) deals with the response of organisms to various factors in the environment.

A Liebig's Law of the Minimum (Figure 1) states that the distribution of a species may be limited by one or more essential environmental factors which occur in minimal quantities.

B Shelford on the other hand pointed out in his Law of Tolerance (Figure 1) that there are also maximum values of most environmental factors which can be tolerated. In between these two extremes there are ranges which may be called "optimum" for factors useful to the organism. Purely deleterious factors on the other hand have a maximum tolerable value, but no optimum. The range between the maximum concentration (greater than zero) which kills no organism and the minimum concentration which kills all organisms is known as the "critical range."

C These principles apply to all aquatic life whether in a stream, lake, estuary, or treatment plant. They are the basis for the control or regulation of biological conditions.

III INDIRECT TOXICITY: MODIFICATIONS OF THE ENVIRONMENT WHICH AFFECT AQUATIC LIFE

A Deposition of inert precipitates and silt tends to smother bottom organisms. Contributing materials include silt or sand from erosion due to poor agricultural practices, rock flour or tailings from mining or quarry operations, mica, coal washings, sawdust and debris from lumbering, insoluble precipitates or complexes from chemical industries.

1 Vulnerable organisms include important fish foods such as insect larvae and snails; also fish eggs, bottom-living algae such as diatoms, and many others.

2 Physical injury to delicate membranes of eyes, and gills may also result.

3 Inert suspended materials and dyes reduce light penetration, suppress photosynthesis and hence biological productivity. They also prevent game fish and other predators from seeing their prey, thereby reducing the efficiency of food utilization.

The word "stream" should be interpreted in most cases to mean "river," "lake," "estuary," etc., as applicable.

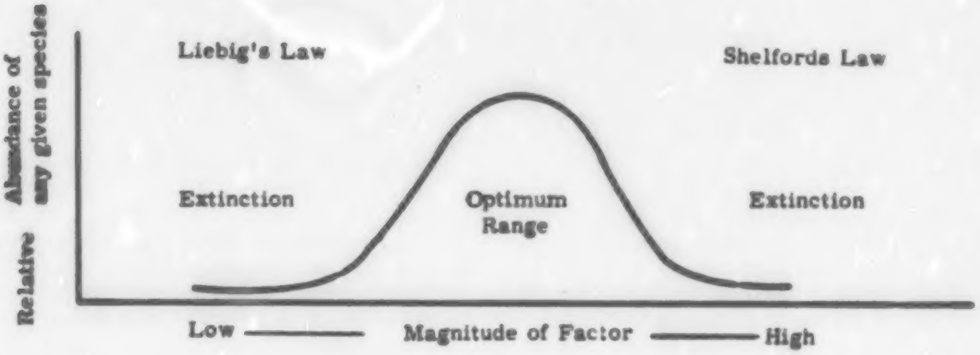


Figure 1. EFFECTS OF ENVIRONMENTAL FACTORS

B Wastes of significant heat content may change the "climate" of a body of water. Temperature may be higher or lower than normal.

1 Abnormally low summer temperatures may prevent the reproduction of some of the typical inhabitants, on the other hand colder water forms such as trout may be enabled to survive.

2 Abnormally high winter temperatures may encourage a rapid development of some species, thus for example, causing an early emergence of some insect followed by its death from normal winter temperatures. Southern forms may also invade more northerly waters, sometimes leading to a year-round nuisance from flying swarms of adults such as caddis flies.

3 Artificially produced high temperatures, often lack dependability. It is discouraging for organisms to spend six days in summer temperatures in January, only to freeze to death over the weekend because no one warned them that the plant would shut down!

4 Excessively high summer temperatures, even for a few hours once a year, probably represent the greatest temperature danger. Some species of fish can adjust to temperatures approaching 100° F, if the change takes place slowly. Each species however, has some maximum temperature above which it cannot survive (Figure 1).

a Quick temperature changes are fatal at much lower values.

b Lack of oxygen due to low solubility at high temperatures or from an increase in the rate of the BOD, also contributes greatly to high temperature mortalities.

C Oxygen-consuming wastes kill by depleting the free dissolved oxygen resources.

1 Amount present, rather than percentage of saturation, is usually more significant.

2 Minimum amounts rather than averages are most critical.

a Any species can survive something less than the optimum concentration of DO for a limited period of time. There is, however, some concentration for any given temperature, which will eventually result in the death of that species. Let us call this the "critical" DO.

b As the DO falls below the critical concentration, survival time eventually drops to zero.

c The absolute values of these thresholds vary with the species and other factors. Five mg O_2 per liter is often listed as a minimum permissible value to maintain a well-rounded healthy population of fishes on a year-round basis.

3 Low oxygen tensions may also increase the toxicity of certain chemicals.

D pH

1 "pH" is a logarithmic expression of the hydrogen ion concentration in a solution. Hydrogen ions ($H+$) in certain concentrations are toxic to aquatic life (as are also hydroxyl ions: OH^-). Aquatic life in relative abundance and variety can be found in waters ranging from approximately pH 5 to 9. Thriving communities including algae, insects, and fish have been studied in waters with a pH of at least 11.

2 Many species of aquatic organisms can adjust to pH values over a wide range. Sudden change of any kind however can be fatal.

3 Most metals and other toxic substances in dilute solution tend to become less toxic at high pH values. A notable exception is ammonia.

IV POLLUTION WHICH RENDERS FISH OR SHELLFISH UNUSABLE OR INTERFERES WITH THEIR CAPTURE

A Radioactivity at levels currently found in our waters has not been observed to adversely affect aquatic life itself. It may however, be taken up with food materials and render fish or fishery products unusable.

1 Radioactive nuclides (forms of chemicals) are taken up by the plants (predominantly algae) in the processes of photosynthesis and other types of protoplasmic synthesis. There is no selection between nuclides on the basis of radioactivity.

2 As the chemicals originally assimilated by the algae are "eaten up the food chain" (from algae to invertebrates to small fish to large fish to man and other predators) their radioactivity moves along with them.

3 Thus radioactivity is acquired by fishes essentially through food, and scarcely at all by direct assimilation or absorption.

4 Plankton-feeding organisms such as herrings, oysters, and clams acquire radioactivity directly from the algae on which they feed. Since they concentrate this food from large volumes of water, they may be much "hotter" than the surrounding water itself.

B Fish may be repelled or driven out of an area by obnoxious chemicals. This may simply result in their scarcity or absence from a given locale, or it may prevent their swimming up a river to spawn. In this case the species would soon disappear and be lost to the community.

C Color, odor, oil, floating scum, bacterial slimes, and other such materials tend to discourage sport fishing and interfere with gear used by commercial fisheries.

D Sublethal concentrations of chemicals such as phenol, benzene,oil, 2-4-D, etc., may impart an unpleasant taste to fish flesh, even when present in very dilute concentrations. This is nearly as detrimental to the fisheries as a complete kill, and of course applies to shellfish as well as fin fish.

E Minamata disease was first described from Minamata, Japan, as a disorder resulting from eating various seafoods taken from Minamata Bay, Kyushu, Japan. The disease results from industrial toxicity, in this case an organic mercury compound, transmitted to a wide variety of local marine seafood species. These organisms are not known to be affected, but acting as "transvectors," pass the toxin along to predators or human consumers. Over 30% mortality occurred in Minamata among people eating local seafood.

1 It may be important to note that a fish kill was recently reported from a TVA lake in this country resulting from mercury leaching from corroded 50 gallon drums used as floats by marinas.

2 Bird and fish kills have recently occurred in Swedish lakes resulting from mercury compounds from pulp mills. Levels in pike exceed the WHO standards for human consumption and the local population has been advised not to eat pike more than once a week! Cases of high mercury levels are increasing.

V DIRECT TOXICITY: AFFECTS THE ORGANISM ITSELF

Fish kills are often the result of direct toxicity. If this is sufficiently potent to kill at once, or within a few days, it is called acute, and is often observed as a "fish kill."

Action that may require weeks or months to be effective may be referred to as low-level, cumulative, or chronic toxicity, and is more often observed as simply a reduction of productivity: "Fishin' ain't what it used to be." Examples of chemicals often believed to be involved include: acids, alkalines, ammonia, chlorine, cyanides, metals, phenols, solvents, sulfides, synthetic organic chemicals, oil field brines, pesticides, herbicides, detergents and others.

A Acute toxicity may be so broadly effective that many forms of life are affected at one time, or it may be highly selective. It may result from a low concentration of a highly toxic material or a high concentration of a relatively less toxic material.

1 It is frequently encountered as a "slug" resulting from a dump or spill, followed by normal, relatively non-toxic conditions as the mass of water containing the poison flows on downstream, or is deflected by tidal movements.

2 Acute toxicity can be evaluated by means of the toxicity bioassay technique and various modifications (Figure 2).

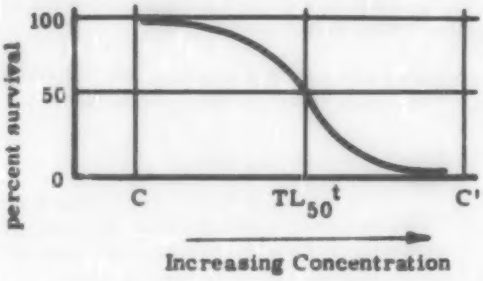


Figure 2 - Critical Range

C: maximum concentration at which no fish die, C': minimum concentration at which all die. TL_{50}^{t}, 50% tolerance limit (concentration tolerable to 50% of the population) for time t.

B Chronic or low-level toxicity may change the entire population balance.

1 Susceptible species of either fish or fish food organisms may die off, thereby permitting tolerant species to flourish for lack of competition.

2 If algae and/or invertebrate food organisms are killed off fish may die or move out of the area.

3 Weakened individuals are more susceptible to attack by parasites and disease, such as the aquatic fungus Saprolegnia.

4 Reproductive potential may be altered. Eggs or fry may or may not be more susceptible to toxic substances than adults.

5 Host fish for mussel (unionidae) life cycle may not survive.

6 The result may be a slow and subtle alteration of the characteristics of a stream over an extended period of time.

C The specific physiological mechanisms involved are infinite in variety and but little known. Included are such processes as enzyme inhibition as in the case with some of the pesticides, and over stimulation of mucous membranes of the gills, leading to death by suffocation.

D There are a number of excellent diagnostic techniques for the examination of dying fish, these include pathogenic bacteria, parasites, some metals, and certain pesticides. None are routine but require specific handling and preservation techniques.

E A recently developed procedure for protecting aquatic life from deleterious substances is biomonitoring. This is the continuous monitoring or surveillance of an effluent for toxicity by means of a system for exposing living organisms (such as fish or invertebrates) to a continuously flowing stream in a dilution just below the known danger point. Should the toxicity of the substance increase the test organisms respond in some recognizable manner, thus giving warning that corrective measures need to be initiated.

VI MECHANISMS OF POLLUTION TOLERANCE AND SENSITIVITY

The fact that some organisms are more resistant to pollution than others needs no emphasis. The matter of "why?" and "how?" on the other hand, is quite another question. In some cases the answer is obvious, in others not. In general we can say that the adaptations of certain species enable them to resist certain types of natural conditions such as organic deposits or sand bars. When man artificially creates conditions such as sludge banks, or sand bars, organisms which can tolerate such conditions move in, survive, and often thrive. Other forms are eliminated.

A Organic pollution is essentially non-toxic. Its typical result as noted above is oxygen depletion, physical turbidity, and smothering blankets of sediment or sludge.

B Devices and mechanisms for living in oxygen-poor or oxygen-free water include the following:

1 Obtaining oxygen from the air by means of periodic trips or access to the surface.

a The snorkel tube of the rat-tailed maggot.

b Periodic trips of the mosquito larvae and Corixidae or water boatmen. The mosquito takes air directly into its respiratory system, the water boatman into a trap or space beneath the wing covers, as well as into a layer of air held by fine hairs or "pile" all over its body.

c Behavior of the air breathing snails such as Physa which have an internal lung cavity.

d Insects which tap into air tubes in aquatic plants.

e Fishes which gulp air at surface or breathe surface water.

2 Special devices and behavior for respiration of water

a Hind intestine respiratory structures of dragonfly larvae permits respiration in silt-laden water.

b Movement of gill covers and similar structures in isopods and certain insect groups maintains a current of water over respiratory organs.

c Body movements of chironomid larvae create water current in tube. Sludge worms and other annelids also create water movement by means of sinuous body movements.

3 Physiological and behavioral adaptations to endure low oxygen tensions.

a Forms possessing accessory respiratory pigments such as hemoglobin might be expected to be able to be able to extract the last vestige of dissolved oxygen from the water. Two groups famous for resisting low DO do have hemoglobin: the larvae of certain Chironomid midge flies, and small annelid worms such as sludge worms. (It should be noted however, that the hemoglobin in each case is simply dissolved in the blood plasma, rather than being concentrated in special corpuscles as is the case in the more efficient vertebrate system.)

b The mere possession of hemoglobin however, does not seem to assure tolerance of low DO (Walshe '47). Larvae of the midge *Tanytarsus spp.* have hemoglobin, but will not tolerate oxygen-poor waters. Hemoglobin-bearing *Chironomus bathopilus* is moderately tolerant, and *Chironomus plumosus* is highly tolerant, however.

c During periods of low DO, *Chironomus plumosus* apparently respires carbohydrates as usual, but excretes excess lactic acid instead of accumulating it.

d Various species of *Daphnia* (microcrustacea) have been shown to accumulate hemoglobin in oxygen-poor waters but not in oxygen-rich waters (Fox '47). No clear adaptive significance has yet been proven however.

C Advantageous Feeding Habits

All highly organic pollution tolerant organisms are scavengers, and hence find an abundance of food. Most are relatively defenseless and hence have normally high reproductive rate. The result in a polluted situation is thus usually an extreme abundance.

D The reverse problem is why are intolerant species intolerant?

1 A physiological requirement for higher oxygen levels is probably most basic.

2 Turbidity would hamper any organisms employing sight in any way.

3 Absence of light would suppress the growth of green algae, and hence also restrict the growth of algae feeders.

E Inert silts by themselves have many damaging effects such as abrasive or smothering action.

Biological mechanisms for enduring inert silt or sand pollution are not numerous, and consequently such locations are usually known as biological deserts. Since some life exists even in deserts however, a few forms may occasionally be found. In general they are typical sand or mud dwellers. Since the available food in such a substrate is at best of a very low order, inhabitants of these situations must either seek buried or trapped food particles or capture food from the passing waters.

1 If there is no BOD involved, and water and oxygen circulate down into the deposit, burrowing forms such as certain mayflies, annelid worms, ammocetes lamprey larvae, microcrustaceans, and others may burrow down to depths of two feet or more. Fish eggs normally deposited in gravel and newly hatched larvae are also dependent on circulating water. Such a population can be killed overnight by a layer of fine sediment or sludge which seals the surface to water circulation.

2 Water or plankton feeders include clams and mussels which can move about freely in a soft, shifting bottom, thus keeping on top of silt or sand as

it accumulates. If deposition is actively taking place, however, there will probably be so much turbidity in the water that plankton (food) organisms are unable to live. Under such circumstances certain clams and snails have the ability to close the shell so tightly by means of valves or an operculum that all contact is lost with the environment for extended periods, during which CO_2 tends to lower the rate of body metabolism. Organisms of this type have been reported to be dug up with sand and gravel and incorporated into concrete products while still alive. Emergence of a population of asiatic clams (Corbicula) for example, just as a big block of concrete is setting is said to be rough on contractors!

3 An interesting situation occasionally develops in estuaries where mud of moderate organic content is slowly deposited over oyster beds. The oysters are unable to move, but as they grow, their shells tend to bend upward above the accumulating silt, and may grow to several inches in length while growing very little in width. Crowding brings about a similar reaction in the effort to avoid being buried.

F Few generalizations can be offered relative to toxic pollution except that toxicity is relative, and all forms do not respond equally to a given toxicant.

1 Few mechanisms of toleration can be listed, beyond the natural resistance that certain forms may have for a given condition. For example, some marine species may survive a salt concentration that is toxic to freshwater species. Over a period of several generations, some species may develop a genetic resistance to some toxicant such as insecticides in the same way that DDT-resistant strains of houseflies have developed. Copper sulfate, and chlorine-resistant strains of algae such as Cosmarium for example, may develop in treated water supply reservoirs.

2 Some bottom in-fauna organisms such as annelid worms may retreat down into burrows until a slug of undesirable water passes.

3 Molluscs may close shells tightly for the same purpose. The metabolic rate is known to diminish with the increase of CO_2 inside the closed shell, thereby enabling them to remain tightly sealed for extended periods of time.

VII EFFECTS OF LIFE HISTORY STAGES

A In order to survive in a polluted area, each life history stage of an indigenous organism must be able to survive in turn.

B If some given life history stage cannot tolerate conditions, and the species is present:

1 Fortuitous changes may occur at the proper time(s) to permit survival of the more susceptible stages(s) or:

2 Recruitment from less polluted areas may occur.

C Some examples of reproductive stages or procedures which might affect pollution sensitivity:

1 Egg or egg-like stages are often enclosed in protective membranes, jelly masses, or cases. May remain dormant until favorable conditions develop.

2 Eggs may be deposited in locations where they are less exposed to polluted water as:

buried in the gravel,
on the surface film,
on rocks over the water moistened by spray,
on mud surface near water,
or in locations where maximum water circulation is encountered as at lip of waterfall.

3 Eggs may require minimal DO due to low metabolic rate.

4 Eggs deposited on or in bottom may be susceptible to smothering.

5 Newly hatched larvae often continue to live on stored yolk material for a time. On beginning to take natural food, they may be killed by toxic content thereof, such as organochlorines.

6 Some forms such as certain sludge worms commonly reproduce by (vegetative) fragmentation, hence avoiding egg and larva stages.

VIII NATURAL SELECTION AND ACCLIMATIZATION TO POLLUTION

A Known biological mechanisms for selective breeding of pollution resistant strains operate in nature among fishes as among other organisms.

1 Studies of population genetics indicate that after some finite number of generations of population stress (e.g., exposure to a given pollutant), permanent heritable resistance may be expected to develop.

2 If the environmental stress (or pollutant) is removed prior to the time that permanent resistance is developed in the population, reversion to the non-resistant condition may occur within a relatively few generations.

3 Habitats harboring populations under stress in this manner are often marked with the dead bodies of the unsuccessful individuals.

B Individual organisms on the other hand can over a period of time (less than one life cycle) develop a limited ability to tolerate different conditions, e.g., pollutants:

1 With reference to all categories of pollutants both relatively facultative and obligate species are encountered (e.g., euryhaline vs stenohaline, eurythermal vs stenothermal).

2 This temporary somatic acclimatization is not heritable.

C A given single-species collection or sample of living fishes may therefore represent one or more types of pollution resistance:

1 A sample of an original population which has been acclimated to a given stress in toto.

2 A sample of the surviving portion of an originalpopulation, which has been "selected" by the ability to endure the stress. The dead fish in a partial fish kill are that portion of the original population unable to endure the stress.

3 A sample of a sub-population of the original species in question which has in toto over a period of several generations developed a heritable stress resistance.

D Any given multi-species field collection will normally contain species illustrative of one or more of the conditions outlined above.

ACKNOWLEDGMENTS:

Certain portions of this outline contain material from a prior outline by Croswell Henderson and revisions by R. M. Sinclair.

REFERENCES

1 Cordone, A. J. and Kelley, D.W. The Influence of Inorganic Sediment on the Aquatic Life in Streams. Calif. Fish and Game. 47:189-228. No. 2. 1961.

2 Ellis, M.M. Detection and Measurement of Stream Pollution. Bull. 22, U.S. Bur. Fish: also, Bull. Burg. Fish 48: 356-437. 1937.

3 Foster, R.F. and Davis, J.J. The Accumulation of Radioactive Substances in Aquatic Forms, No. A/Conf. 8/P/280. U.S.A. International Conf. on Peaceful Uses of Atomic Energy. pp. 1-7. 1955.

4 Ingram, W.M. and Towne, W.W. Stream Life Below Industrial Outfalls. Public Health Reports. 74:1059-1070. 1959.

5 Kurland, Leonard. The Outbreak of a Neurologic Disorder in Minimata, Japan, and its Relationship to the Ingestion of Seafood Contaminated by Mercuric Compounds. Proc. Nat. Shell. Sanit. Workshop. pp. 226-228. 1961.

6 Mackenthun, K.M. and Keup, L.E. Assessing Temperature Effects with Biology. Proc. Am. Power Conf. Vol. 31. pp. 335-343. 1969.

7 Tarzwell, C.M. and Gaufin, R.R. Some Important Biological Effects of Pollution Often Disregarded in Stream Surveys. Purdue Univ. Engr. Bull. Proceedings 8th Ind. Waste Conf. May 4, 5, and 6, 1953.

8 Tarzwell, C.M. Hazards of Pesticides to Fishes and the Aquatic Environment. The Use and Effects of Pesticides. Proc. of Symposium, Albany, N.Y. Sept. 23, 1963. N.Y. State Joint Legis. Comm. on Nat. Resources, Albany, N.Y. pp. 30-40.

Vinson, S.B., Boyd, C.E. and Ferguson, D.E. Resistance to DDT in the Mosquito Fish *Gambusia affinis* Science. 139:217-218. January 18, 1963.

10 Walshe, Barbara M. On the Function of Hemoglobin in *Chironomus* after Oxygen Lack. Jour. Exp. Biol. Cambridge. 124:329-342. 1947.

SUPPLEMENTARY READING

1 Bullock, Glen L. A Schematic Outline for the Presumptive Identification of Bacterial Diseases of Fish. Prog. Fish Cult. 23(4):147-151. 1961.

2 Foster, R.F. and Davis, J.J. Aquatic Life Water Quality Criteria. Second Progress Report, Aquatic Life Advisory Committee, Sewage and Ind. Wastes, 28:678-690. 1956.

3 Fox, H. Munro. Daphnia Hemoglobin. Nature. London. p. 431. September 27, 1947.

4 Ingram, W.M. and Wastler, III, T.A. Estuarine and Marine Pollution. Selected Studies, U.S. DHEW, PHS, Robt. A. Taft Sanitary Engineering Center, Cincinnati, Ohio. Technical Publication No. W6 1-04.

5 Jackson, H.W. and Brungs, Wm. A. Biomonitoring of Industrial Effluents. Purdue Industrial Waste Conference, Layfayette, Indiana. May 3-5, 1966.

6 Rodhe, W. Limnology, Social Welfare, and Lake Kinneret. Int. Jour. Limnology, Vol. 17. November 1969.

7 Tennessee Valley Authority, Fish Kill in Boone Reservoir. TVA Water Qual. Branch. Chattanooga, Tenn. 1968.

8 Robert A. Taft Sanitary Engineering Center. Pesticides in Soil and Water. An annotated Bibliography. PHS Publication No. 999-WP-17. September 1964.

9 Stewart, R. Keith, Ingram, William M. and Mackenthun, Kenneth. Selected Biological References on Fresh and Marine Waters. FWPCA Publication No. WP-23, pp. 126. 1966.

10 Warren, Charles E. Biology and Water Pollution Control. W.B. Saunders Co. 434 pp. 1971.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, OWP, EPA, Cincinnati, OH 45226.

NUTRIENTS: THE BASIS OF PRODUCTIVITY

I INTRODUCTION

A Nutrients of importance include macronutrients: those needed in large quantities, and micronutrients: those needed in small amounts.

B These nutrients are important because they promote biological responses which may interfere with some desired use of the water by man.

C Other factors (e.g. temperature, light) affect the use of these nutrients and should be considered in an evaluation of the effects of nutrients upon the ecosystem.

II Algae, bacteria, fungi and aquatic plants are the forms of life which nutrients affect most directly.

A Algae are of Several Types

1 Phytoplankton are small algae suspended in the water and form the basis of productivity in the aquatic environment.

2 Benthic algae are those forms anchored to substrates of rock and bottom materials.

B Aquatic plants are of several types. In general they may be referred to as rooted or floating forms.

C Heterotrophic bacteria are fungi which respond to organic nutrients introduced into water. Autotrophic bacteria may respond and grow due to inorganic nutrient sources.

III BIOLOGICAL LAWS

A Liebig's "law" of the minimum: the essential material available in amounts most closely approaching the critical minimum needed will tend to be the limiting factor.

B Shelford's "law" of tolerance: survival of an organism can be controlled by the quantitative or qualitative deficiency or excess with respect to any one of several factors which may approach the limits of tolerance for that organism.

C Q_{10} "law": with a temperature increase of 10 degrees centigrade metabolic processes (rates) are approximately doubled.

IV The process of photosynthesis is the fixation of the sun's energy with the production of organic matter by plants with chlorophyll.

A The general reaction is given below:

$$CO_2 + H_2O \xrightarrow{\lambda} CH_2O + O_2$$

B Chlorophyll contains basically C, O, H, N and Mg, and in general makes up about 5% of the dry weight of algal cells.

V MEASUREMENT OF PHOTOSYNTHESIS

A Oxygen production can be used as a measure of photosynthesis because for each mole of CO_2 reduced to organic carbon one mole of free oxygen is liberated.

1 The value of the molar O_2/CO_2 ratio has been found experimentally to vary within wide limits.

B CO_2 Assimilation

1 The CO_2 taken up by algae does not all originate from the dissolved gas. Some algae can use bicarbonate directly as a source of carbon.

2 Hence measurement of CO_2 uptake from water is a complicated problem which must consider pH, HCO_3^-, and $CO_3^=$ concentrations.

C Fixation of Carbon-14

1 The use of C^{14} as a tracer of C^{12} in plant metabolism and productivity estimation has been widely used since the early nineteen fifties.

2 In this method a known amount of C^{14} is added to the water and after a period of time the proportion of C^{14} in the plant cells to C^{14} added is found. The amount of carbon assimilated is then estimated from the following equation.

$$\frac{\text{activity of phytoplankton}}{\text{activity of } C^{14}O_3^{=} \text{ added}} (K) = \frac{\text{total carbon assimilated}}{\text{total carbon available}}$$

3 Where K is a constant relating to the slower uptake of C^{14}.

4 The total carbon available is determined chemically.

D Uptake of Mineral Nutrients

1 The measurement of depletion of nutrients in solution has been tried but found unreliable.

E Chlorophyll

1 The quantity of chlorophyll present has been found to bear some relation to productivity but not a reliable one.

VI Nutrients of significance in the growth and production of algae and plants are discussed below.

A Carbon

1 Sources

a Gaseous CO_2

b HCO_3^-

c $CO_3^=$

d Other carbon compounds

2 Effects of the removal of carbon upon the water

a Lowered pH

b Deposition of $CaCO_3$

3 The quantity of carbon available is great and it usually is not a limiting factor.

B Nitrogen

1 Nitrogen can be taken up by most algae as either ammonium salts or as nitrates. Nitrites can also be used but a high concentration is usually inhibitory. Some blue green algae can fix atmospheric nitrogen. Certain algae varieties require supplementary amines, growth factors, etc.

2 The quantity of nitrogen in waters has definitely been shown to limit algal populations.

C Phosphorus

1 Phosphate seems to be the only inorganic source of this nutrient.

2 Limiting concentrations of P have been found to range from .01 ppm at a minimum and an inhibitory affect if P concentrations exceed 20 ppm.

3 Optimum concentrations have been found to range from .018 ppm to 15 ppm.

4 Storage of inorganic phosphate by algae has been demonstrated. The extent of this storage may reach 80% of the total phosphorus in algal cells.

D Silicon

1 Nutrient ratios in the algal cells of some areas have been found to be Si 23: N16: P1. It can be seen from this ratio that silicon is an important element in algal growth.

2 Silicon is especially important in the population growth of diatoms and may be the limiting growth factor in these populations.

E Inorganic micronutrients - Many elements are needed in very small quantities by algal cells. Some of these have a known function in algal metabolism; others do not.

1 Mg is a cation of major importance in the chlorophyll molecule.

2 Co is known to be necessary for vitamin B_{12}.

3 Mn is necessary for several enzyme systems.

4 Mo, V, Zn, and Cu are necessary but these functions are not as well known.

F Organic Micronutrients

1 Of 179 algal strains investigated about 40% required vitamin supplementation for optimum growth. Principal growth factors that were not synthesized in sufficient quantity are given as follows along with the percentage of the vitamin deficient strains showing marked productivity gain after supplementation:

a B_{12} addition increased growth on 80% of the strains.

b Thiamin addition increased growth on 53% of the strains.

c Biotin addition increased growth on 10% of the strains.

2 Algae can use and may require many organic compounds depending upon environmental conditions and the ability of the organism to synthesize required building blocks from mineral forms of C, N, & P. This is an area for continued investigation with many unappreciated or vaguely understood ecological factors.

VII PROBLEMS AND BENEFITS RESULTING FROM ALGAE PRODUCTION

A Problems to man may result when the total "primary production" by algae leads to an increase in the total organic content of the water that interferes with a desired use.

1 This may consist of a high algal population that produces a water with high turbidity, taste and odor, or other undesirable effect. High respiratory needs may lead to nocturnal oxygen deficit.

2 Certain algae may cause tastes and odors, clog filters; or otherwise interfere with potable water processing.

3 Death of large algal populations may lead to tastes and/or odors through bacterial decomposition. Oxygen deficits may result at any time of day in this process. Deposition of masses of organic sediment or sludge may be considerable.

4 Other problems might be cited.

B The primary production of algae can also serve as a supply of food to consumer organisms (animals), resulting in increased production at several (trophic) levels:

zoömicrobes, microinvertebrates, macroinvertebrates, fishes.

1 Earlier notation cited the release of oxygen during utilization of CO_2 during algal photosynthesis. This encourages fungal or bacterial breakdown of pollutants.

2 Photosynthesis occurs in the presence of adequate light and favorable conditions. In darkness, the cells continue to respire and may consume more oxygen than they produced because photosynthesis increases the organic load.

3 Photosynthesis tends to occur at the surface where light intensity is greatest. Poor vertical mixing would result in stratification of water supersaturated with oxygen over oxygen deficient water. Depending upon conditions, a significant fraction of the oxygen could be lost to the atmosphere.

4 Increased productivity may result in temporary reduction of the free dissolved nutrient level in the water but harvesting at some level is essential to prevent later recycle.

VIII CYCLE OF NUTRIENTS

A Once nutrients enter a body of water they are cycled through a food chain.

B Factors affecting this food chain (e.g. toxicity, removal) will affect the concentration and distribution of the nutrients.

ACKNOWLEDGEMENT:

This outline contains certain material submitted by F. J. Ludzack and H. W. Jackson.

REFERENCES

1 Golterman, H. L. and Clymo, R. S. Chemical Environment in the Aquatic Habitat (Proc. of an IBP - symposium, Amsterdam and Nieuwersluis Oct. 1966). 322 pp. (N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam. 8.95).

2 Lewin, Ralph A. Physiology and Biochemistry of Algae. Academic Press. 1962.

3 Odum, Eugene P. Fundamentals of Ecology. W. B. Saunders Co. 1959.

4 Odum, H. T. Primary Production in Flowing Waters. Limnology and Oceanography. 1(2):102-117. April 1956.

5 Ryther, John H. The Measurement of Primary Production. Limnology and Oceanography. 1(2):72-84. April 1956.

6 Verduin, Jacob. Primary Production in Lakes. Limnology and Oceanography. 1(2):85-91. April 1956.

7 Symposium. Factors That Regulate the Wax and Wane of Algal Populations. Inter. Assoc. of Theor. and Appl. Biol. Communications No. 19. 1971.

This outline was prepared by Michael E. Bender, Biologist, Formerly with Training Activities, Ohio Basin Region, SEC.

BIOLOGICAL ASPECTS OF NATURAL SELF PURIFICATION

I INTRODUCTION

A The results of natural self purification processes are readily observed. Did they not exist, sewage (and other organic wastes) would forever remain, and the world as we know it would long ago have become uninhabitable. Physical, chemical, and biological factors are involved. The microscopic and macroscopic animals and plants in a body of water receiving organic wastes are not only exposed to all of the various (ecological) conditions in that water, but they themselves create and profoundly modify certain of those conditions.

B Since toxic chemicals kill some of or all of the aquatic organisms, their presence disrupts the natural self purification processes, and hence, will not be considered here. The following discussion is based solely on the effects of organic pollution such as sewage or other readily oxidizable organic wastes.

C This description is based on the concept of a "stream" since under the circumstances of stream or river flow, the events and conditions occur in a linear succession. The same fundamental processes occur in lakes, estuaries, and oceans, except that the sequence of events may become telescoped or confused due to the reduction or variability of water movements.

D The particular biota (plants and animals, or flora and fauna) employed as illustrations below are typical of central United States. Similar or equivalent forms occur in similar circumstances in other parts of the world.

E This presentation is based on an unpublished chart produced by Dr. C.M. Tarzwell and his co-workers in 1951. Examples from this chart are employed in the presentation.

II THE STARTING POINT

A A normal unpolluted stream is assumed as a starting point. (Figure 1)

B The cycle of life is in reasonably stable balance.

C A great variety of life is present, but no one species or type predominates.

D The organisms present are adjusted to the normal ranges of physical and chemical factors characteristic of the region, such as the following:

1 The latitude, turbidity, typical cloud cover, etc. affect the amount of light penetration and hence photosynthesis.

2 The slope, cross sectional area, and nature of the bottom affect the rate of flow, and hence the type of organisms present deposition of sludge, etc.

3 The temperature affects both certain physical characteristics of the water, and the rate of biological activity (metabolism).

4 Dissolved substances naturally present in the water greatly affect living organisms (hard water vs. soft water fauna and flora).

E Clean water zones can usually be characterized as follows:

1 General features:

a Dissolved oxygen high

b BOD low

c Turbidity low

d Organic content low

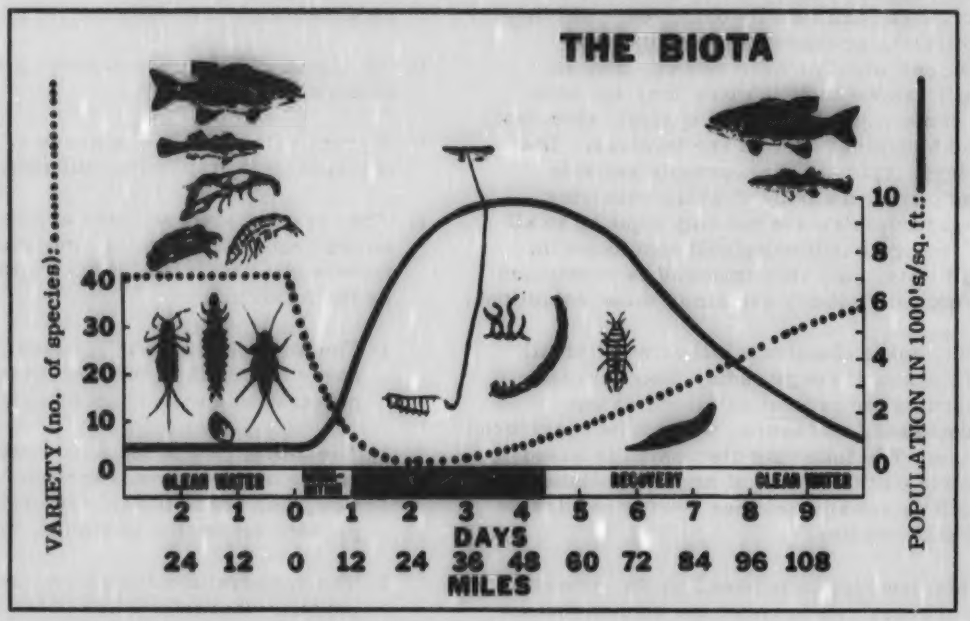


Figure 1: Relations between variety and abundance (production) of aquatic life, as organic pollution (discharged at mile 0) is carried down a stream. Time and distance scales are only relative and will be found to differ in nearly every case. After Bartsch and Ingram.[1]

e Bacterial count low

f Numbers of species high

g Numbers of organisms of each species moderate or low

h Bottom free of sludge deposits

2 Characteristic biota includes a wide variety of forms such as:

a A variety of algae and native higher (vascular, or rooted) plants

b Caddis fly larvae (Trichoptera)

c Mayfly larvae (Ephemeroptera)

d Stonefly larvae (Plecoptera)

e Damselfly larvae (Zygoptera)

f Beetles (Coleoptera)

g Clams (Pelecypoda)

h Fish such as:

- Minnows (Notropid types)
- Darters (Etheostomatidae)
- Millers thumb (Cottidae)
- Sunfishes and basses (Centrarchidae)
- Sauger, yellow perch, etc. (Percidae)
- Others

3 Organisms characteristic of clean lakes, estuaries, or oceanic shores might be substituted for the above, and likewise in the following sections. However, it should be recognized that no single habitat is as thoroughly understood in this regard as the freshwater stream.

III POLLUTION

A With the introduction or organic pollution (Figure 1, day 0), a succession of fairly well organized events is initiated. Important items to observe in interpreting the pollutional significance of stream organisms are the following:

B Numbers of species present, they tend to decrease with pollution.

C Numbers of individuals of each species tends to increase with pollution.

D Ratios between types of organisms are disturbed by pollution.

1 Clean water species intolerant of organic pollution tend to become scarce and unhealthy.

2 Animals with air breathing devices or habits tend to increase in numbers.

3 Scavengers become dominant

4 Predators disappear

5 Higher plants, green algae, and most diatoms tend to disappear.

6 Blue green algae often become conspicious

E The importance of observations on any single species is very slight.

IV THE ZONE OF RECENT POLLUTION

A The zone of recent pollution begins with the act of pollution, the introduction of excessive organic matter: food for microorganisms (Figure 1, day 0)

B There follows a period of physical mixing.

C Many animals and plants are smothered or shaded out by the suspended material.

D With this enormous new supply of food material, bacteria and other saprophytic microorganisms begin to increase rapidly.

E The elimination of intolerant predatory animals allows the larger scavengers to take full advantage of the situation.

F This explosive growth of organisms, particularly fungi and bacteria, draws heavily on the free dissolved oxygen for respiration, and may eventually eliminate it.

G The number of types of organisms diminishes but numbers of individuals of tolerant types may increase.

H Zone of degeneration, or recent pollution, can usually be characterized as follows:

1 General features:

a DO variable, 2 ppm to saturation

b BOD high

c Turbidity high

d Organic content high

e Bacterial count variable to high

f Number of species declines from clean water zone

g Number of organisms per species tends to increase

h Other: Slime may appear on bottom

2 Characteristic biota:

a Fewer higher plants, but rank heavy growth of those which persist

b Increase in tolerant green, and blue green algae

c Midge larvae (Chironomidae) may become extremely abundant

d Back swimmers (Corixidae) and water boatmen (Notonectidae) often present

e Sludge worms (Tubificidae) common to abundant.

f Dragonflies (Anisoptera) often present have unique tail breathing strainer

g Fish types, eg:

- Fathead minnows (Pimephales promelas)
- White sucker (Catostomus commersonni)
- Bowfin (Amia calva)
- Carp (Cyprinus carpio)

V THE SEPTIC ZONE

A The exact location of the beginning of the septic zone, if one occurs, varies with season and other circumstances. (Figure 1, day 1)

B Lack of free DO kills many microorganisms and nearly all larger plants and animals, again replenishing the mass of dead organic material.

C Varieties of both macro and micro-organisms and adjustable types (facultative) that can live in the absence of free oxygen (anaerobic) take over.

D These organisms continue to feed on their bonanza of food (pollution) until it is depleted.

E The numbers of types of organisms is now at a minimum, numbers of individuals may or may not be at a maximum.

F The septic zone, or zone of putrefaction can usually be characterized as follows:

1 General features:

a Little or no DO during warm weather

b BOD high but decreasing

c Turbidity high, dark; odoriferous

d Organic content high but decreasing

- e Bacterial count high
- f Number of species very low
- g Number of organisms may be extremely high
- h Other: Slime blanket and sludge deposits usually present, oily appearance on surface, rising gas bubbles

2 Characteristic biota:

- a Blue green algae
- b Mosquito larvae
- c Rat-tailed maggots
- d Sludge worms (Tubificidae and similar forms). Small, red, segmented (annelid) worms seem to be characteristic of this zone in both fresh and salt waters, the world around.
- e Air breathing snails (*Physa* for example)
- f Fish types: None

3 Note: Fortunately, all polluted waters do not always degenerate to "septic" conditions.

VI THE RECOVERY ZONE

A The septic zone gradually merges into the recovery zone. (Figure 1, day 4)

B As the excessive food reserves diminish so do the numbers of anaerobic organisms and other pollution tolerant forms.

C As the excessive demand for oxygen diminishes, free DO begins to appear and likewise oxygen requiring (aerobic) organisms.

D As the suspended material is reduced and available mineral materials increase due to microbial action, algae begin to increase often in great abundance.

E Photosynthesis by the algae releases more oxygen, thus hastening recovery.

F Since algae require oxygen at all times for respiration (like animals), heavy concentrations of algae will deplete free DO during the night when it is not being replenished by photosynthesis.

G Consequently this zone is characterized by extreme diurnal fluctuations in DO.

H With oxygen for respiration and algae, etc. for food, general animal growth is resumed.

I The stream may now enter a period of excessive productivity which lasts until the accumulated energy (food) reserves have been dissipated.

J Zone of recovery may usually be characterized as follows:

1 General features:

- a DO 2 ppm to saturation
- b BOD dropping
- c Turbidity dropping, less color and odor
- d Organic content dropping
- e Bacterial count dropping
- f Numbers of species increasing
- g Numbers of organisms per species decreasing, (with the increase in competition)
- h Other: Less slime and sludge

2 Characteristic biota

- a Blue green algae
- b Tolerant green flagellates and other algae
- c Rooted higher plants in lower reaches
- d Midge larve (Chironomids)

e Black fly larvae (Simulium)

f Giant water bugs (Belostoma spp.)

g Clams (Megalonais)

h Fish types:

- Green sunfish (Lepomis cyanellus)
- Common sucker (Catostomus commersonni)
- Flathead catfish (Pylodictis olivaris)
- Stoneroller minnow (Campostoma anomalum)
- Buffalo (Ictiobus cyprinellus)

3 Excessive production and extreme variability often characterize middle and lower recovery zones.

4 Unfortunately, many waters once polluted never completely "recover". Re-pollution is the rule in many areas so that after the initial pollution, clear out delineation of zones is not possible. Characterization of these waters may involve such parameters as productivity, BOD, some "index" figure, or other value not included here.

VII CLEAN WATER ZONE

A Clean water conditions again obtain when productivity has returned to a normal, relatively poor level, and a well balanced varied flora and fauna are present. (Figure 1, day "10") Conditions may usually be characterized as follows:

B General features: similar to upstream clean water except that it is now a larger stream.

C Characteristic biota: similar to upstream clean water fauna and flora except that species include those indigenous to a larger stream.

REFERENCES

1 Bartsch, A.F. and Ingram, W.M. Stream Life and the Pollution Environment. Public Works Publications, July 1959, Vol. 90, No. 7, pp. 104-110.*

2 Gaufin, A.R. and Tarzwell, C.M. Aquatic invertebrates as indicators of stream pollution. Reprint No. 3141 from PHR. 67 (1):57-64. 1952.

3 Gaufin, A.R. and Tarzwell, C.M. Environmental changes in a polluted stream during winter. Am. Midland Naturalist. 54:68-88. 1955.

4 Gaufin, A.R. and Tarzwell, C.M. Aquatic macro-invertebrate communities as indicators of organic pollution in Lytle Creek. Sewage and Ind. Wastes. 28:906-24. 1956.

5 Hynes, H.B.N. The Biology of Polluted Waters. Liverpool Univ. Press. pp. 202. 1963.

6 Katz, M. and Gaufin, A.R. The effects of sewage pollution on the fish population of a midwestern stream. Trans. Am. Fisheries Soc. 82:156-65. 1952. *

7 Reish, D.J. The Relationship of the Polychaetous Annelid Capitella capitata (Fabricius) to Waste Discharges of Biological Origin. In: Biol. Prob. Water Pol. - Trans. 1959 Seminar. Robert A. Taft Sanitary Engineering Center, USPHS, Cincinnati, OH. pp. 195-200.

8 Biology of Water Pollution FWQA Pub. CWA-3 (references with an asterisk are reprinted in this publication. 1967.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

THE EFFECTS OF POLLUTION ON LAKES

I INTRODUCTION

The pollution of lakes inevitably results in a number of undesirable changes in water quality which are directly or indirectly related to changes in the aquatic community.

A Industrial Wastes may contain the following:

1 Sewage

2 Dissolved organics--synthetics, food processing wastes, etc.

3 Dissolved minerals--salts, metals (toxic and nontoxic), pigments, acids, etc.

4 Suspended solids--fibers, minerals, degradable and non-degradable organics

5 Petroleum products--oils, greases

6 Waste heat

B The Materials in Domestic Wastes which affect Water Quality are:

1 Pathogenic fecal microorganisms

2 Dissolved nutrients: minerals, vitamins, and other dissolved organic substances

3 Suspended solids (sludge)--degradable and non-degradable organic materials

C Pollution and Eutrophication

The discharge of domestic wastes often renders the receiving water unsafe for contact water sports and water supplies. For example, some beaches on the eastern seaboard and in metropolitan regions of the Great Lakes are unfit for swimming because of high coliform counts. Other effects of domestic pollution include changes in the abundance and composition of populations of aquatic organisms.

1 As the nutrient level increases, so does the rate of primary production.

2 Shore-line algae and rooted aquatics become more abundant. For example, problems have been experienced with *Cladophora* and *Dichotomosiphon* along the shores of Lakes Ontario, Erie, and Michigan. These growths interfere with swimming, boating, and fishing, and cause odors when the organisms die and decay.

3 The standing crop of phytoplankton increases, resulting in higher counts and greater chlorophyll content. Increases in phytoplankton abundance may result in taste and odor problems in water supplies, filter clogging, high turbidity, changes in water color, and oxygen depletion in the hypolimnion.

4 Populations of fish and larger swimming invertebrates increase, based on the increase in basic food production.

5 Changes in dominant species

a Diatom communities give way to blue-greens. Toxic blue-greens may pose a problem.

b Zooplankton changes include replacement of *Bosmina coregoni* by *B. longirostris*.

c Trout and whitefish are replaced by perch, bass, and rough fish.

d Hypolimnion becomes anaerobic in summer; bottom sludge buildup results in loss of fish food organisms, accompanied by increase in density of sludgeworms (oligochaeta).

II HISTORICAL REVIEW

The cultural eutrophication of a number of lakes in Europe and America has been well documented.

A Zurichsee, Switzerland

1 1896 - sudden increase in *Tabellaria fenestrata*

2 1898 - sudden appearance of *Oscillatoria rubescens* which displaced *Fragilaria capucina*

3 1905 - *Melosira islandica* var. *helvetica* appeared

4 1907 - *Stephanodiscus hantzschii* appeared

5 1911 - *Bosmina longirostris* replaced *B. coregoni*

6 1920
1924 - *O. rubescens* occurred in great quantities

7 1920 - milky-water phenomenon; precipitation of $CaCO_3$ crystals (40μ) due to pH increase resulting from photosynthesis

8 Trout and whitefish replaced by perch, bass, and rough fish

B Hallwilersee, Switzerland

1 1897 - *Oscillataria rubescens* not observed up to this time

2 1898 - *O. rubescens* bloomed, decomposed, formed H_2S, killing off trout and whitefish

C Lake Windermere, England (core study)

1 Little change in diatoms from glacial period until recent times

2 Then *Asterionella* appeared, followed by *Synedra*

3 About 200 years ago, *Asterionella* again became abundant

4 *Asterionella* abundance ascribed to domestic wastes

D Finnish Lakes

Aphanizomenon, *Coelosphaerium*, *Anabaena*, *Microcystis*, are the most common indication of eutrophy.

TABLE 1 CHANGES IN PHYSIO-CHEMICAL PARAMETERS

Zurichsee, Switzerland

Parameter	Date	Value
Chlorides	1888	1.3 mg/l
	1916	4.9 mg/l
Dissolved organics	1888	9.0 mg/l
	1914	20.0 mg/l

		Max.	Min.
Secchi Disk	before 1910	16.8M	3.1M
	1905 - 1910	10.0M	2.1M
	1914 - 1928	10.0M	1.4M
Dissolved oxygen, at 100 M, mid-summer	1910 - 1930	Minimum	100% saturation
	1930 - 1942	"	9% saturation

E Linsley Pond, Connecticut

1 Species making modern appearance include Asterionella formosa, Cyclotella glomerata, Melosira italica, Fragilaria crotonensis, Synedra ulna

2 Asterionella formosa and Melosira italica were considered by Patrick to indicate high dissolved organics

3 Bosmina coregoni replaced by B. longirostris

F Lake Monona, Wisconsin

1 Began receiving treated sewage in 1920, developed blue-green algal blooms.

G Lake Washington, Washington

1 1940 - Bosmina longirostris appeared

2 1955 - Oscillatoria rubescens seen for the first time, and constituted 96% of phytoplankton, July 1

H Lake Erie

1 Phytoplankton counts at Cleveland have increased steadily from less than 500 cells/ml in the 1920's to over 1500 cells/ml in the 1960's

2 Abundance of burrowing mayflies (Hexagenia spp.) in Western Lake Erie decreased from 139/m^2 in 1930, to less than 1/m^2 in 1961.

I Lake Michigan

1 Milky water observed in south end, and in limnetic region in mid-1950's and again in 1967.

2 During the period 1965-1967 the Chicago water treatment plant has found it necessary to increase the carbon dosage from 23 lbs/mil gal to 43 lbs/mil gal, and the chlorine dosage from 20 lbs/mil gal to 25 lbs/mil gal.

3 Phytoplankton counts in the south end now exceed 10,000/ml during the spring bloom.

III FACTORS AFFECTING THE RESPONSE OF LAKES TO POLLUTION INCLUDE:

A Depth-surface area ratio: A large hypolimnion will act as a reservoir to keep nutrients from recirculating in the trophogenic zone during the summer stratification period. Rawson found an inverse relationship between the standing crop of plankton, benthos, and fish, and the mean depth.

B Climate: Low annual water temperatures may restrict the response of the phytoplankton to enrichment.

C Natural color or turbidity: Dystrophic (brown-water) lakes may not develop phytoplankton blooms because of the low transparency of the water.

IV TROPHIC LEVEL

Except in cases where massive algal blooms occur, the trophic status of lakes is often difficult to determine. Core studies are used to determine trends in diatom populations which might indicate changes in nutrient levels over an extended period of time.

V CONTROL OF POLLUTION

The success of efforts to arrest the eutrophication process, and where desirable, reduce the trophic level of a lake, will depend on a thorough knowledge of the nutrient budget.

A Significant quantities of nutrients may enter a lake from one or more of the following sources:

1 Rainfall

2 Ground water

TABLE 2 PARAMETERS COMMONLY USED TO DESCRIBE CONDITIONS

	Oligotrophic Condition
1 Transparency	≥ 10 meters
2 Phosphorus	≤ 1μg/l
3 NO_3 - Nitrogen	≤ 200 μg/l
4 Minimum annual hypolimnetic oxygen concentration	near 100% saturation
5 Chlorophyll	≤ 1 mg/m^3
6 Ash-free weight of seston	≤ 0.1 mg/l
7 Phytoplankton count	≤ 500/ml
8 Phytoplankton quotients	
a $\frac{\text{number of species of Chlorococcales}}{\text{number of species of Desmids}}$	<1
b $\frac{\text{Myxophycease+Chlorococcales+Centrales+Euglenaceae}}{\text{Desmidaceae}}$	<1
c $\frac{\text{Centrales}}{\text{Pennales}}$	0 - 0.2
9 Phytoplankton species present (see outline on plankton in oligotrophic lakes).	

3 Watershed runoff

4 Shoreline domestic and industrial outfalls

5 Pleasure craft and commercial vessels

6 Waterfowl

7 Leaves, pollen, and other organic debris from riparian vegetation

B The supply of nutrients from "natural" sources in some cases may be greater than that from cultural sources, and be sufficient to independently cause a rapid rate of eutrophication regardless of the level of efficiency of treatment of domestic and industrial wastes.

C Many methods have been employed to treat the symptoms, reduce the eutrophication rate, or completely arrest and even reverse the eutrophication process.

1 Use of copper sulfate, sodium arsenite, and organic algicides: It is not economically feasible to use algicides in large lakes.

2 Addition of carbon black to reduce transparency. This is likewise frequently impractical.

3 Harvesting algae by foam fractionation or chemical precipitation.

4 Reducing nutrient supply by (a) removal of N and P from effluents, (b) diversion of effluents, and (c) dilution with nutrient-poor water.

D Examples of lakes where control has been attempted by reducing the nutrient supply, are:

1 Lake Washington, Seattle

The natural water supply for this lake is nutrient poor ($Ca = 8$ mg/l, $P \leq 5$ μg/l, $TDS = 76$ mg/l). Since the turnover time of the water in this lake is only three years, it was expected that diversion of sewage would result in a rapid improvement of water quality. Diversion began in 1963, and improvements were noticeable by 1965 - including an increase in transparency, and a reduction in seston, chlorophyll, and epilimnetic phosphorus.

TABLE 3

PHOSPHORUS REDUCTION IN LAKE WASHINGTON

Year	Maximum phosphorus in upper 10 meters (μg/l)
1963	70
1964	66
1965	63

2 Green Lake, Washington

The lake has a long history of heavy blooms of blue-green algae. Beginning in 1959, low-nutrient city water was added to the lake, reducing the concentration of phosphorus by 70% in the inflowing water. By 1966, the lake had been flushed three times. Evidence of improvement in water quality was noted in 1965, when Aphanizomenon was replaced by Gleotrichia.

3 Lake Tahoe

This lake is still decidedly oligotrophic. To maintain its high level of purity, tertiary treatment facilities were installed in the major sewage treatment plant, and construction is now underway to transport all domestic wastes out of the lake basin.

REFERENCES

1 Ayers, J.C. and Chandler, D.C., Eds. Studies on the environment and eutrophication of Lake Michigan. Special Report No. 30. Great Lakes Research Division, Institute of Science and Technology, University of Michigan, Ann Arbor. 1967.

2 Brezonik, P.L., Morgan, W.H., Shannon, E.E., and Putnam, H.D. Eutrophication factors in North Central Florida Lakes. University of Florida Water Res. Center. Pub. #5, 101 pp. 1969.

3 Carr, J.F., Hiltunen, J.K. Changes in the bottom fauna of Western Lake Erie from 1930 to 1961. Limnol. Oceanogr. 10(4):551-569. 1965.

4 Frey, David G. Remains of animals in Quaternary lake and bog sediments and their interpretation. Schweizerbartsche Verlagsbuchhandlung. Stuttgart. 1964.

5 Edmondson, W.T., and Anderson, G.C. Artificial eutrophication of Lake Washington. Limnol. Oceanogr. 1(1):47-53. 1956.

6 Fruh, E.G. The overall picture of eutrophication. Paper presented at the Texas Water and Sewage Works Association's Eutrophication Seminar, College Station, Texas. March 9, 1966.

7 Fruh, E.G., Stewart, K.M., Lee, G.F., and Rohlich, G.A. Measurements of eutrophication and trends. J.W.P.C.F. 38(8):1237-1258. 1966.

8 Hasler, A.D. Eutrophication of lakes by domestic drainage. Ecology 28(4):383-395. 1947.

9 Hasler, A.D. Cultural Eutrophication is Reversible. Bioscience 19(5): 425-443. 1969.

10 Herbst, Richard P. Ecological Factors and the Distribution of *Cladophora glomerata* in the Great Lakes. Amer. Midl. Nat. 82(1):90-98. 1969.

11 National Academy of Sciences. Eutrophication: Causes, Consequences, Correction. 661 pp. 1969. (Nat. Acad. Sci.,2101 Constitution Avenue, Washington, DC 20418, 13.50).

12 Neel, Joe Kendall. Reservoir Eutrophication and Dystrophication following Impoundment. Reservoir Fisheries Res. Symp. 322-332.

13 Oglesby, R.T. and Edmondson, W.T. Control of Eutrophication. J.W.P.C.F. 38(9):1452-1460. 1966.

14 Stewart, K.M. and Rohlich, G.A. Eutrophication - A Review. Publication No. 34, State Water Resources Control Board, The Resources Agency, State of California. 1967.

This outline was prepared by C.I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

SIGNIFICANCE OF EUTROPHICATION

I INTRODUCTION

A Eutrophication may be defined briefly as enrichment (of the aquatic environment) leading to the production of aquatic life. It is part and parcel of the process of natural self-purification.

B Productivity is defined briefly as the ability of water (or land) to produce a crop of living things.

C A baseline assumption of the present discussion is the absence of toxicity.

II NATURAL EUTROPHICATION

A Eutrophication may occur naturally in the course of geologic time.

B The classic example is a "pot-hole" lake formed when a large block of glacial ice (which had been buried in sand and gravel) melted.

1 At the outset, it was a barren hole in a vast gravel plain filled with ice water.

2 Life quickly became established as bacteria and algae drifted in. The nutrient base at this time was the inorganic dissolved solids in water.

3 As time went on, dead organic matter began to accumulate on the bottom as ooze. Rooted vegetation crept in around the shore. Leaves, sticks, and other dead organic matter accumulated around the margins until a mat of sod and peat began to build out from the shores. This overgrowth continued until the water was closed over and bog resulted. Bog grew into solid soil and the lake disappeared forever.

4 The processes of eutrophication therefore led to the self-elimination of the lake by means of organic matter essentially produced within its own confines and the immediate environs.

C All natural eutrophication does not necessarily lead to the elimination of water basins; other geologic processes often intervene. But it is interesting to note that vast quantities of carbon, hydrogen, and oxygen have been immobilized in the earth's crust as coal and peat measures as the result of eutrophication and biological productivity in the Carboniferous (late Paleozoic). Streams may have a natural productivity resulting from materials leached from bed rock or other materials.

III EUTROPHICATION BY MAN

A Covert (Concealed) Eutrophication via Soil Seepage

1 This is a slow but sure route for much nutrient material deposited in or on the ground to reach open water.

2 A limited(?) and unknown amount of production occurs in the subterranean environment. This includes the growth of soil bacteria and other fungi, or animals, and does not generally result in the entrainment of additional energy content in the biomass (mass of organic substance).

3 Due to the generally low rate of water seepage through soil, visible eutrophication of open bodies of water by this route is likely to be slow to develop, and equally slow to dissipate, should the source be eliminated.

4 Examples could be cited from Florida to Washington and Maine to California. Sources of the nutrient are generally seepage from agricultural crop fertilization, or soil absorption from individual sewage disposal systems.

B Overt (Open) Eutrophication

1 Raw waste discharges of sewage, dairy wastes, food processing wastes, etc.

a Likely to be conspicuous, intermittent, and obnoxious.

b Often create nuisance zones in streams or lakes during which mineralization takes place.

c Environmental eutrophication cannot begin until mineralization has proceeded to a point where basic plant nutrients are available, although very heavy "consumer" growth (scavenger animals) may result from direct feeding on the waste itself.

2 Treated waste discharges

a Generally less environmentally traumatic than raw waste; nuisance zones less common.

b Conventional secondary treatment less damaging than primary treatment alone.

c Residual minerals and energy content still inevitably available for eutrophication.

3 Overt eutrophication generally more remediable and subject to control than covert.

a Waste flow is completely controllable (barring accidents), as compared to diffuse seepage through soil.

b The quantity of eutrophic material in a body of water at any given time is finite and degradable. Given time, without replenishment, it will be exhausted.

IV SIGNIFICANCE OF EUTROPHICATION

A A point of view such as a water quality objective, for example, is necessary for the evaluation of eutrophication.

1 Enrichment of a water supply reservoir is generally considered bad, as it usually leads to the development of blooms of plankton organisms causing tastes and odors, or the development of an anaerobic hypolimnion with accompanying H_2S and other problems.

2 Enrichment of a fishing lake is considered good to the point where maximum fish production can be obtained.

B Eutrophication exists in many degrees; a little may be good, too much may be bad.

C A little natural productivity (eutrophication) plus a little man-made productivity may lead to too much total productivity.

D Eutrophication is inexorable and inevitable. Once the basic ingredients have been dumped into a body of water, somewhere, sometime, they are sure to be assimilated by an organism and thus contribute to new growth. Burial in a sludge bank or discharge on a flood simply delays the result in time or changes the location.

E Deliberately encouraged, eutrophication may constitute a final stage in treatment.

V CONCLUSION

Eutrophication is neither good nor bad, but thinking makes it so! (Apologies to Shakespeare)

REFERENCE

Stewart, Kenton M. and Rohlich, Gerald A. Eutrophication - A Review. Publication No. 34, California State Water Quality Control Board, p 188. (1967)

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

POLLUTION OF THE MARINE ENVIRONMENT

I Estuaries are the hydrologic exit points for inland lakes and streams and the oceans are the receiving waters.

The accumulated pollution load of the entire central portion of the country is disgorged through them.

A Estuarine waters are thus essentially "river waters" near their heads merging seaward into typically marine waters. Mixtures of fresh and salt water are called "brackish."

B Bays and the open ocean are essentially similar in water characteristics but usually have distinctive patterns of water circulation.

C Approximately one third of the population of the U.S. (over 55 million people) lives on estuaries, and seven of the worlds ten greatest cities are on estuaries.

II MARINE WATERS AS RECEIVING WATERS

A Marine waters are extremely "hard," as compared to most inland waters. The total dissolved solids content is measured in terms of parts per thousand (‰) rather than parts per million.

1 Water from the open ocean ranges from 33-35 parts per thousand of total salinity by weight (33,000-35,000 ppm).

2 There is considerable readjustment of the dissolved solid ratios as inland waters mix with seawater.

a Some of the solids, suspended in river water, may be dissolved in oceanic water, whereas other materials may be precipitated and dropped as silt. There is thus a certain amount of "natural" siltation unrelated to man-made pollution.

b It has recently been established that much of the silt in certain estuaries is of oceanic origin. In others it is of river origin.

c Biological assimilation removes additional material from solution.

B There is a great (but not inexhaustible) capacity for dilution in the oceans.

1 Though we will probably never approach the assimilative capacity of the open oceans as a whole for the natural purification of sewage type wastes, severe local disturbances of the natural ecological balance (pollution) are legion in coastal areas.

2 Safe dilution limits for radioactive wastes appear to be definitely limited.

a The effects of conditions in abyssal depths on waste storage containers cannot currently be predicted.

b Oceanic water masses are known to turn over and major currents to change at long or presently unpredictable intervals.

c Long half-life radioactive isotopes could accumulate dangerously in the biota.

III EFFECTS OF ORGANIC OR SEWAGE-TYPE POLLUTION

These materials while detrimental, can eventually be metabolized by biological processes.

It should be noted that many brackish water forms, particularly those adapted to life in brackish marshes and tidal flats, are highly tolerant of variable conditions, often naturally including high organic content. These then might be expected to tolerate moderate amounts of organic pollution and could be listed as "facultative."

A Molluscs

1 Oysters (such as Crassostrea virginica) and mussels (such as Mytilus edulis) are attached and unable to move to overcome silt accumulation.

  a Slow siltation results in upward elongation of existing individuals.

  b Continuous accumulation of silt will prevent the attachment of young larvae.

  c Organic pollution is usually sewage which not only eliminates oysters and mussels ecologically, but also renders them unacceptable for sanitary reasons.

  d They seem to be able to tolerate considerable intermittent oxygen depletion.

2 The term "clam" includes such forms as the hardshell clam or quahog (Mercenaria mercenaria) and the softshell clam (Mya arenaria) of the Atlantic, and razor clam (Siliqua patula) and geoduck (Panope generosa) of the Pacific coasts.

  a Being unattached and naturally active burrowers, these animals are able to overcome sheer siltation.

  b Their sensitivity to oxygen depletion resembles that of the oyster.

3 Since clams and oysters are plankton feeders, it should be noted that they are particularly liable to the accumulation of radioactive wastes.

  a Some radioactive isotopes are assimilated by certain algae and phytoplankton.

  b As the bivalves feed on the plankton, these radioactive chemicals will in turn be assimilated by them.

  c Details have yet to be worked out, but preliminary investigations have demonstrated the basic facts.

4 Some snails have known responses to pollution and other ecological factors.

  a The mud snail (Nassa spp.) is often found on shallow tidal mud flats with high temperatures and high organic content.

  b The European snail (Bulla stricta) is listed as being favored by pollution.

B Annelids

A type of annelid worm known as sludgeworms are found to be favored by severe organic pollution, even though anaerobic conditions may obtain. These are represented in freshwater by such genera as Tubiflex and Limnodrillus and in seawaters by Spio fulginosus, Capitella capitata and others.

1 Due to their possession of hemoglobin they are able to extract the last trace of available oxygen from polluted water, and there is good evidence that at least some of them may utilize bound oxygen under anaerobic conditions.

2 A variety of annelid worms often dominates the fauna of a polluted area, even when industrial wastes are prevalent.

3 Members of this group apparently feed actively on the organic mud or sludge itself.

C Heavy organic pollution will eliminate the macrofauna and flora of an estuary or ocean front, as in a freshwater river or lake, largely as a result of oxygen

Table II - Pollution Tolerance of Marine Organisms
(As reported by Von Wilhelm, 1916 and Mohr, 1952)

	Response to Pollution		
	Intolerant	Facultative	Favored
THALLOPHYTES			
Chlamydothrix			xx
Enteromorpha spp.		x	
Ulva spp.		x	
PROTOZOA (Ciliophora)			
Eutreptia			x
Vorticella (Campannla type)			x
Epistylis			x
Vaginicola			x
Euplotes			x
Stylonychia			x
Lacrymaria			x
PLATYHELMINTHES			
Plagiostoma girardi			xx
MOLLUSCS			
Bornia corbuloides			x
Capsa frigilis			x
Tapes aureus			x
Bulla stricta			x
Dirois verrucosa			x
Spurilla neapolitans			x
Mytilus edulis		x	
Cardium edule		x	
ANNELIDS			
Spio fulginosus			xx
Capitella capitata			xx
Arenicola claparedei & A. grubei			x
Hydroides pectineta & H. uncinata			x
Spirographis salanganis			x
Staurocephalua rudolphi			x
Sternaspis thalassimoides			x
ECHINODERMS			
Asterias tennispina		x	
BRYOZOA			
Bugula avicularia & B. calathus			x
Bugula purpurotincta			x
CRUSTACEA			
Nebalia golatea			x
Brachynotus sexdentatus			x
Balanus spp.		x	
TUNICATES			
Cione intestinalis			x
Botryllus aurolineatus			x
Box salpa		x	

x - indicates general degree of tolerance
xx - strongly favored

deficiency, or the physical accumulation of a sludge blanket on the bottom. Living matter, however, is represented by bacteria and molds. The impoundment of inactive water over such an area hastens this destructive process.

1 As distance up or down the estuary provides time and dilution, biological oxidation and mineralization proceeds to the point where some elements of the macrofauna and flora can live.

2 These tolerant species, such as, those noted above, then take advantage of the increased concentration of nutrients and the lack of competitors to grow in size and numbers far beyond the natural production levels.

3 This then, is the same expression of recovery from organic pollution found in freshwater, namely: super abundance of life, but generally restricted variety.

D Marine fishes respond to pollution in essentially the same way as freshwater fishes.

1 Due to their being accustomed to moving about in larger bodies of water, they have more chance to avoid the proximity of local pollution sources.

2 Elimination of bottom organisms tends to discourage bottom feeding fish, although scavengers such as sculpins toadfish, and others may often be found in the vicinity of outfalls.

3 Organic sedimentation may directly smother the eggs of bottom spawning fishes such as smelt.

4 Severe pollution in a river-mouth or estuary may also serve as a block to sensitive anadromous fish seeking to run upstream to spawn.

5 Organic enrichment may provide more food and hence increase productivity on the fringes of polluted zones as in freshwaters.

6 Fish such as tarpon and mullet have been observed to thrive in highly enriched brackish water ponds and bays in Florida and elsewhere. This of course is essentially the same type of situation as a fertilized fish pond.

IV TOXIC POLLUTION

The physiological nature of the action of toxicants on organisms is infinitely varied and but partially understood. The adjustment of organisms to varying salinities may in some cases be related to toxicity through the phenomenon of osmosis.

A Organisms may be grouped with reference to their ability to endure salinity changes (Figure 1).

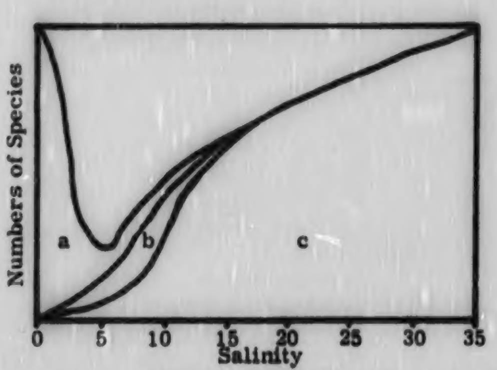


Figure 1. DISTRIBUTION OF ORGANISMS IN AN ESTUARY

a Euryhaline, freshwater
b Indigenous, estuarine, (mesohaline)
c Euryhaline, marine

1 Stenohaline organisms can endure relatively little variation in salinity.

2 Euryhaline organisms can endure considerable variation in salinity.

3 Freshwater organisms having a body containing fluid of a higher osmotic pressure than that of the surrounding media must constantly resist the tendency of that media to penetrate and dilute their body fluids.

4 Marine organisms having a body containing fluid closely resembling the surrounding media, is either in osmotic equilibrium with his surroundings, or may even have to take in extra water to prevent dehydration.

5 Osmotic differences associated with the salinity gradient may play a significant role in determining the toxicity of a given waste at different parts of an estuary. Detergent, for example, has been found to be more toxic in marine waters than in freshwater.

B Outfalls discharging toxic wastes may often be recognized by completely denuded environs.

C Toxic wastes are sometimes eliminated from the water by complexing on suspended material. When this material is deposited, the toxicants may be removed from the waters, but they are deposited in the substrate. Such a situation may often be recognized by a pauperate bottom infauna or epifauna, while the overlying water shows no biological effect. Gradual recovery of the bottom fauna usually takes place with increasing distance from the outfall, provided there are no complicating factors.

D Pollution dilution by sewage. A situation has been observed where the generally toxic waters of an estuary are diluted by the outfall of a domestic sewage treatment plant to such an extent that a limited fauna is able to survive in the vicinity of the sewage outfall.

V SOME PRACTICAL CONSIDERATIONS OF MARINE AND ESTUARINE WASTE DISPOSAL

A Due to the lesser specific gravity of sewage, it has a strong tendency to channel its way to the surface of salt water, with relatively little mixing enroute unless very well diffused at the point of discharge. On the surface it is susceptible to wind drift. If this is onto a bathing beach from an offshore outfall, sanitary conditions may quickly become intolerable.

B Of major significance is the absence of a continuous unidirectional current to remove material from the outfall and immediately begin mixing and dilution. This is of course likewise true of lakes.

1 Oceanic surface currents may be strongly wind influenced, or associated with tidal cycles.

2 Currents in bays and estuaries are at best tidal. Use is sometimes made of this by constructing temporary holding basins and then dumping only on selected phases of the tidal cycle.

3 Estuarine circulation patterns are affected by several factors.

a Tides impart an "in and out" motion to the water (as mentioned elsewhere).

b Inflowing freshwater imparts an "out motion." The resultant of these two is known as the "net tidal drift."

c Typically, cross sectional areas increase as the sea is approached. This reduces the net tidal drift.

d The denser sea water tends to flow in underneath the lighter freshwater in the mixing process.

e The coriolis effect pulls moving bodies to the right (in the northern hemisphere). Although this is insignificant in most river situations, it results in generally lower salinities along the right hand (facing the ocean) shores of estuaries which are not too irregular in shape, as the river water moves down, and higher salinities along the left hand shores as the sea water moves in. This can be seen in such situations as the Chesapeake Bay in Maryland.

f Irregularities of bottom and shore and wind action all combine to complicate and confuse the results of these forces.

4 Careful study of local hydrographic circulation will sometimes reveal other ways in which these phenomena can be put to advantage.

C The physical difficulty of constructing offshore oceanic outfalls which will withstand the rigors of the storm, ice, and shifting sand renders this an extremely expensive undertaking.

REFERENCES

1 Conference on Estuaries. Proceedings. (In Press) Jekyll Island, Georgia. March 31 - April 4, 1964.

2 Daugherty, F. M., Jr. Effects of Some Chemicals Used in Oil Well Drilling on Marine Animals. Sewage and Industrial Wastes. 23 (10):1282-87. October 1951.

3 Galtsoff, Paul S., Chipman, W. A., Jr., Engle, J. B., and Hasler, A. D. Preliminary Report on the Cause of the Decline of the Oyster Industry of the York River, Va., and the Effects of Pulp-Mill Pollution on Oysters. Bureau of Fisheries Investigational Report No. 37. Vol. II. 1938. 42 pp.

4 Hedgpeth, Joel W. Treaties on Marine Ecology and Paleolcology. Geological Society of America. Memoir 67. 1957.

5 Ketchum, Bostwick H. Hydrographic Factors Involved in the Dispersion of Pollutants Introduced into Tidal Waters. J. Boston Soc. Civil Engineer. 37(3):296-314. July 1950.

6 Filice, F.P. The Effects of Wastes on the Distribution of Bottom Invertebrates in the San Francisco Bay Estuary. Ibid. 17:1-17. 1959.

7 Pritchard, D. W. A Review of Our Present Knowledge of the Dynamics and Flushing of Estuaries. Chesapeake Bay Inst. of the Johns Hopkins Univ. Tech. Report 4. Ref. 52-7. March 1952.

8 Reish, D.J. and Winter, H.A. The The Ecology of Alamitos Bay, California with Special Reference to Pollution. California Fish and Game. 40(2):105-121. April 1954.

9 Ingram, W. M. and Doudoroff, P. Publication on Industrial Wastes Relating to Fish and Oysters. U.S. Department of Health, Education, and Welfare. Public Health Service. Publication No. 270.

10 Von Wilhelm, Julius. Synopsis of the Biological Examination of Water. Stizungsberichte der Geslischaft Naturforschende Freunde Nr. 9. November 1916.

11 Saxton, W. W. and Young A. Investigation of Sulfite Wastes Liquor Pollution in Fidalgo and Padilla Bays. Pollution Control Commission Report. Wash. (Undated) (25 mimeographed pages)

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Cincinnati, OH 45268.

WATER TEMPERATURE AND WATER QUALITY

I INTRODUCTION

A Temperature is the basic variable in water "climates".

1 Temperature, or the amount of thermal energy present, is originally of solar or cosmic origin. Biological processes acting over geologic time temporarily capture and store much energy in organic substance. The "fossil fuels" (oil, gas, and coal) which we burn today release solar energy captured in the geologic past, which but for man might not have been released until sometime in the geologic future. Most of the solar energy being captured today, is released probably today, with the possible exception of some unrecognized fraction which is proceeding into long term storage.

The release of atomic energy is of inorganic or cosmic origin, and the magnitude and significance of the additional thermal discharge to the environment has yet to be accessed.

One last observation is important before turning to the details of water temperature and water quality. While we are most critically occupied with the immediate or local impact of a concentration of thermal energy released at a given point in the environment (the excess or "waste" which man is unable to capture and entrain in his electric transmission lines), it should be remembered that of every ton of coal or pound of uranium burned as fuel, nearly 100% of the energy contained eventually finds its way into the general global environment. A small remaining fraction is rebound as chemical energy in some "product."

2 Direct solar radiation is the overriding contributor of thermal energy to all lands and waters.

a Total energy from insolation onto "spacecraft earth" is counterbalanced by the radiation of terrestrial energy into space. If the two do not exactly balance on an annual basis, the overall temperature (climate) of the earth will rise or fall.

b The annual climate or heat budget of a given body of water is determined by its geographic location (latitude elevation, etc.) interacting with local meteorological conditions, and other factors.

c There is, therefore, a natural or normal temperature regimen for any given body of water to which it will tend to return if disturbed by man.

d There is, also, a normal or characteristic community of aquatic organisms that will tend to persist. When the heat budget or climate of a body of water is changed, the fauna and flora change.

3 There is great diversity of opinion, even among knowledgeable people, as to the effects of thermal changes in waters. Some of the reasons for this follow:

a Only a continuously maintained temperature of 100° C could keep a surface water mass sterile. The question is therefore not: life or no-life; but rather: what kind of life is the objective?

b Aquatic life has received more attention than other water uses because the aquatic organisms cannot escape the water conditions.

c There are certain circumstances in which a modest rise in temperature might be considered to be beneficial as for example: keeping an area of a river or a harbor free of ice for navigation, or winter fishing. There has, also, been investigation of the use of warmed waters for certain aspects of aquaculture.

d It is, therefore, important in discussing the virtues and vices of thermal changes to clearly define or specify the objective or type of aquatic community in mind.

4 It is clear that the need for more and more power will continue into the foreseeable future (Table 1).

B Human activities which may modify receiving water temperatures include the following:

1 Logging and other land stripping activities which increase the rate of surface run-off and hence raise or lower temperatures of influent waters, depending on the season.

2 Removal of stream bank shade

3 Erosion which fills in stream bed and causes water to be spread in broad shallow layer, exposed to sun and air.

4 Release of cold waters from hypolimnion of deep reservoirs.

5 Withholding or augmenting flow by dam manipulation.

6 Release of relatively large volumes of high temperature wastewaters from power production and/or industrial processes.

II THERMAL ELECTRIC POWER PRODUCTION AS A STREAM WARMING ELEMENT

A Production of electric power by stream plants involves the wastage of considerable quantities of energy in cooling waters. Approximately 5000 BTU of heat are wasted for each kilowatt of electricity generated. This represents an efficiency of roughly 40%.

B It is estimated that approximately 80% of all energy required in the future will come from steam generating plants.

C Weirs and jetties help greatly in the dispersal of warmed waters, but must be carefully designed to each situation.

D As water temperature rises, its value as a coolant diminishes.

E Heat dissipation from a body of water which has been heated above its equilibrium temperature with the meteorological conditions follows Newton's Law of Cooling which states that the rate of cooling is proportional to the difference between the temperature of the body of water, and the equilibrium temperature for the given meteorological conditions. For example, an analysis of the Ohio River as at Cincinnati has shown that it would require over 200 miles to dissipate 99 + % of heat added (Figure 1).

III EFFECTS OF HEAT ON ORGANIC WASTE DISPOSAL

A Higher temperatures accelerate the rate of bacterial growth, the optimum temperature being in the range of 30° C (86° F).

1 As water temperatures approach this value, the rate of BOD thus approaches a maximum.

TABLE 1

MAXIMUM TEMPERATURES PROBABLY COMPATIBLE WITH THE WELL-BEING OF VARIOUS SPECIES OF FISH AND THEIR ASSOCIATED BIOTA IN °C

Temperature	Taxa
34 C	Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, gizzard shad
32 C	Growth of largemouth bass, drum, bluegill, crappie
29 C	Growth of pike, perch, walleye, smallmouth bass, sauger, California killifish, topsmelt
27 C	Spawning and egg development of catfish, buffalo, threadfin shad, gizzard shad, California grunion, opaleye, northern swellfish
24 C	Spawning and egg development of large-mouthed bass, white and yellow bass, spotted bass, sea lamprey, alewife, striped bass
19 C	Growth or migration routes of salmonoids and for egg development of perch, small-mouth bass, winter flounder, herring
12 C	Spawning and egg development of salmon and trout (other than lake trout)
9 C	Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon

2 If the waste assimilative capacity of a stream is being utilized based on a given stream temperature, and the temperature subsequently raised, the DO may drop so low as to produce fish kills and other nuisance conditions.

B Higher water temperatures may, also, lead to a higher concentration of bacteria pathogenic to man.

IV EFFECTS OF HEAT ON FISH AND OTHER AQUATIC LIFE

A Vulnerability of fish and other aquatic life to high temperatures represents a major restriction on the discharge of cooling water.

1 Involves duration of exposure as well as absolute thermal level.

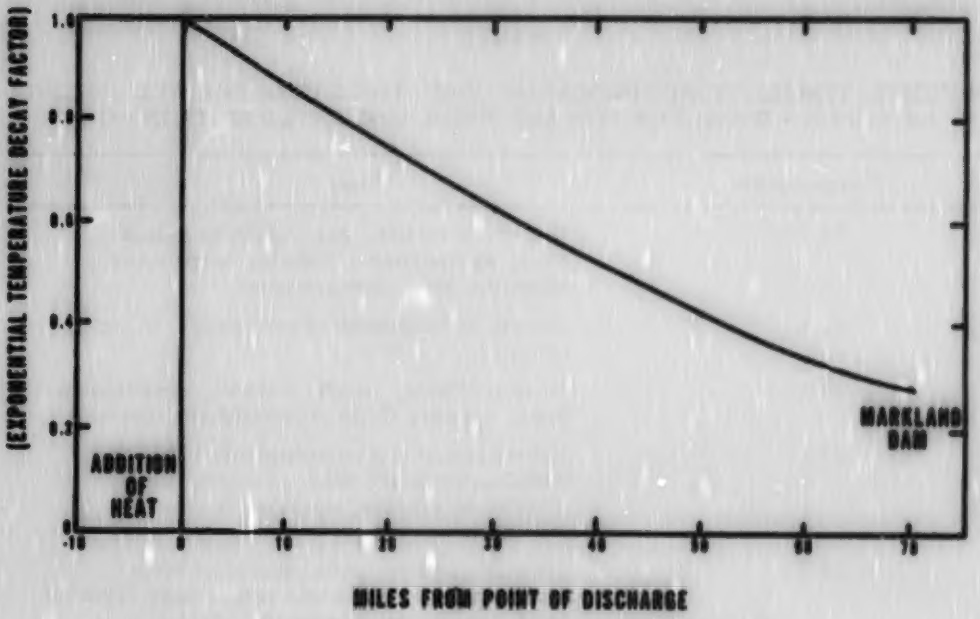
2 Sensitivity to toxic substances is increased.

3 Lower temperatures are required in winter than in summer.

B Factors contributing to the sensitivity of fish to heat

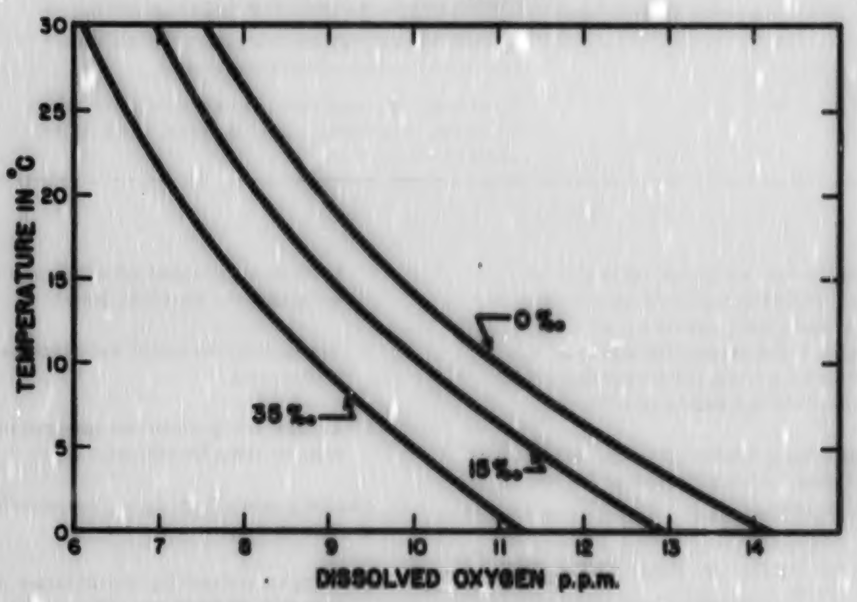
1 Oxygen solubility diminishes as temperature rises (Figure 2).

2 Oxygen requirements of aquatic life increase as temperature rises (Figure 3).



TEMPERATURE DROP THROUGH MARKLAND POOL
OHIO RIVER

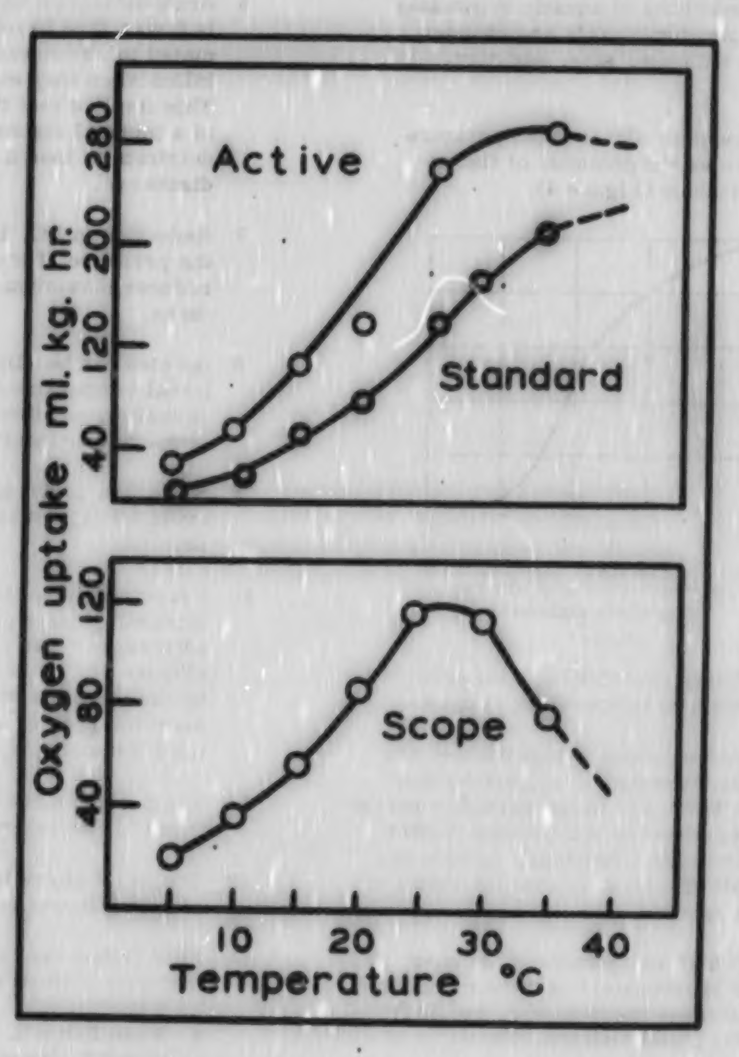
Figure 1



OXYGEN SOLUBILITY AT SELECTED SALINITIES

RICHARDS AND CORWIN, LIMNOLOGY AND OCEANOGRAPHY, 1956 51.223.0c.2.58

Figure 2

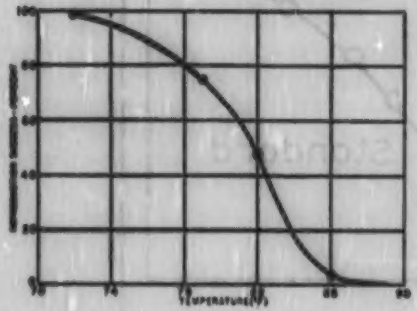


THE RELATION OF TEMPERATURE TO ACTIVE AND STANDARD METABOLISM IN YOUNG GOLDFISH OF AN AVERAGE WEIGHT OF 2 GM. From Fry and Hart (1948)

Figure 3

3 The sensitivity of aquatic organisms to temperature levels and changes varies with age, size, and size.

a A constant elevated temperature reduces the potential of fish to reproduce (Figure 4).



EFFECTS OF CONSTANT TEMPERATURE ON REPRODUCTION OF A MINNOW (*Pimephales promelas*)

Figure 4

b Different species have different preferred temperature ranges.

c Seasonal cooler temperatures are often essential to egg production and hatching, while warmer summer temperatures will promote faster growth after hatching, up to some limit of tolerance characteristic of the species.

4 Lethal high temperatures as determined in laboratory tests vary widely for different species, e.g. goldfish: 107.6° F, pink salmon: 75° F.

a Lethal temperatures differ at different times of the year, as well as for the different life history stages (Figure 5).

b This is analogous to a temperature of 50° F for man: in winter it feels "warm," in summer it is "cool."

5 Sudden changes in water temperature can be fatal to certain organisms, both fish and fish food organisms.

6 Acclimatization to higher temperatures is faster than to lower. Fish acclimated to warm water are rapidly killed when they swim into cold water. This implies that the sudden shutdown of a thermal discharge may be more detrimental than a continuous normal discharge.

7 Reduction in DO, increase in CO_2, or the presence of toxic materials reduces maximum tolerable temperatures.

8 Species can be eliminated at less than lethal temperatures by predators, parasites, or diseases which are less temperature-sensitive.

9 Some fish do not seem to be able to avoid killing hot waters, while others do.

10 Preferred temperature ranges in laboratory tests generally are somewhat higher than in field observations (Figure 6). This may be influenced by the demands of the natural environment for greater activity and hence a need for more oxygen (Figure 2, Table 1).

11 Temperature can act as a directive force in fish migration.

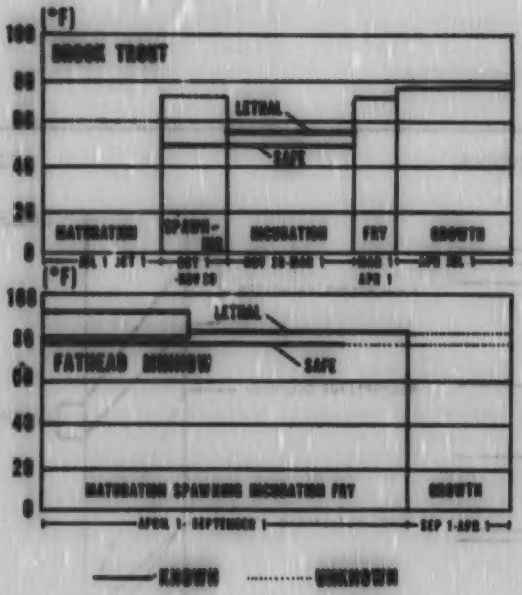
12 The exact physiological mechanisms of heat kill are not fully understood.

Fats rather than proteins seem to be the most critical substance.

a Some fish will die at temperatures of 65° F, lower than that at which proteins usually coagulate.

b Tropical (or heat-adapted) plants and animals often have fats with a higher melting point than arctic (or cold-adapted) organisms.

c Animals (such as goldfish) fed high melting point fats (e.g. beef) develop higher melting point body fats than those fed on low-melting fats (such as fish oil). They are in turn able to tolerate higher temperatures.



THERMAL TOLERANCE OF CRITICAL LIFE HISTORY STAGES

Figure 5

d Lethal temperatures seem to destroy fat-calcium relationships.

C Effects of Temperature on Fish Food Organisms

1 Species composition and abundance are affected in ways similar to the fishes as outlined above.

2 Warm waters encourage blue-green algae. Some can tolerate as high as 185° F for limited periods (Figure 7).

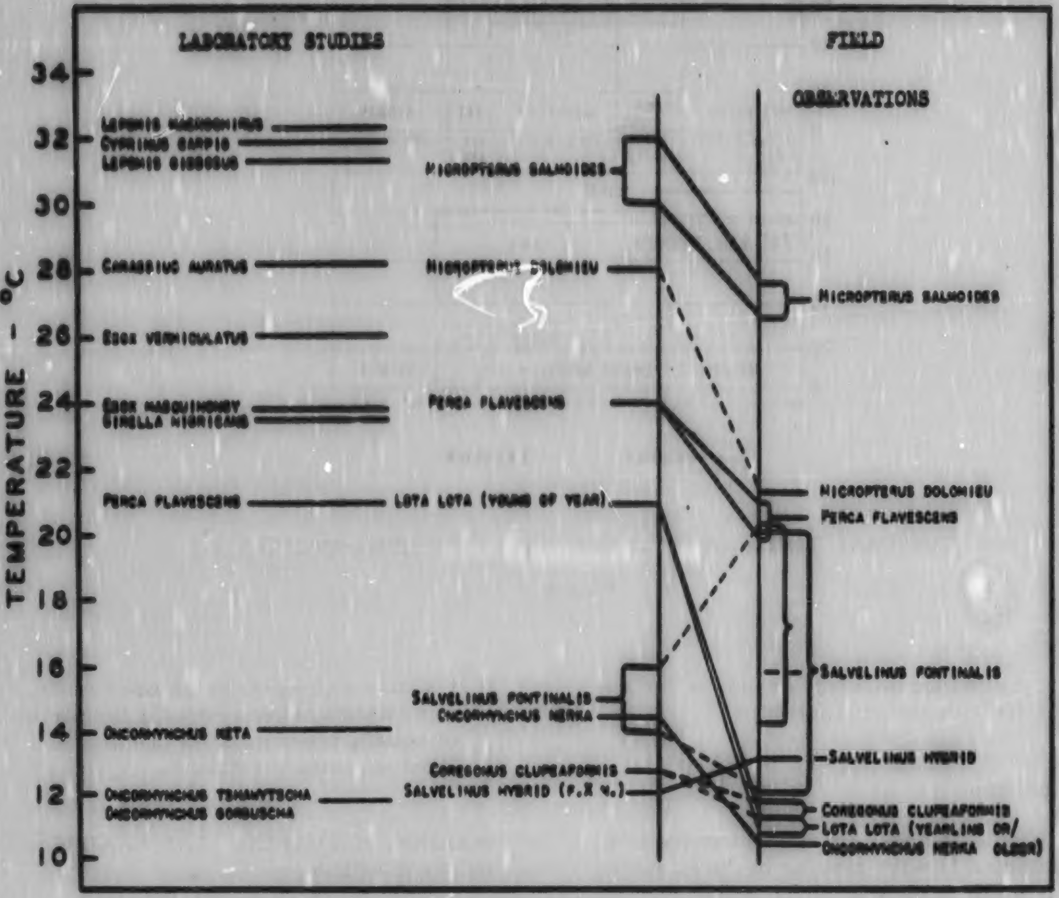
3 A temperature increase of 8° C stimulated photosynthesis in phytoplankton in one series of observations when ambient water temperatures were 16° C or cooler, but inhibited photosynthesis when natural waters were 20° C or warmer. The existence of a diurnal response to thermal stimulation was noted at 9:00 A.M.

4 Warmed waters speed up life cycles and encourage year round emergence of aquatic insects, often creating local nuisance conditions.

V MARINE, ESTUARINE, AND ANADROMOUS SPECIES

A The general principles of biotic responses to thermal conditions outlined above apply as well to salt water forms as to fresh.

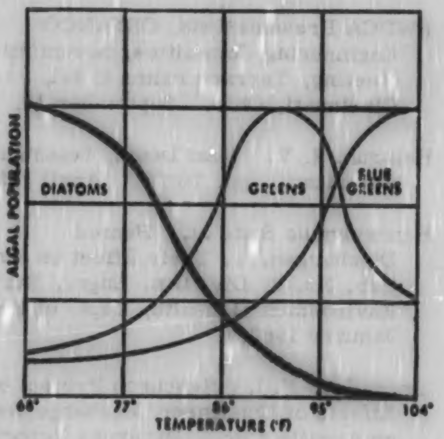
B Salmon do not feed during the spawning migration, hence higher temperatures may so increase their metabolic demands as to deplete their food reserves before spawning can occur. A thermal block at the mouth of a river (e.g. 21.1° C at the mouth of the Okawogan River in Washington) can prevent an entire spawning run.



Left. A comparison of various final preferenda as found by laboratory studies.

Right. A comparison of field observations with laboratory results for a number of species.

Figure 6



EFFECT OF TEMPERATURE ON TYPES OF PHYTOPLANKTON

Figure 7

1 The American Oyster Crassostrea Virginica may spawn, depending on its condition, at temperatures from 15 to 34° C, spawning being triggered by a rise in temperature.

2 The shore crab Carcides Maenas thrives, but does not breed at temperatures between 14 and 28° C. Breeding can only take place outside the heated area.

C Fish in the estuarine environment are more susceptible to temperature changes than those in fresh water. However, wider ranges of tolerance between species exist.

D Most shellfish (in the broad sense: mollusca and crustaceans) are relatively or highly stenothermal (unadaptable to rapid temperature changes). Some are stenothermal for one stage (e.g. spawning), and eury-thermal for others (e.g. growing).

Preliminary observation, also, indicates that heat stress may stimulate oysters to accumulate copper (as other stressful factors are known to do) without being a direct killing agent.

E The distribution of many benthic invertebrate organisms is temperature dependent (See Table 2).

ENVIRONMENTAL TEMPERATURE RANGES OF SOME MARINE INVERTEBRATES

Table 2

Taxa	Temperature range in ° C
American Oyster	4 - 34
European Oyster	0 - 20
Opossum Shrimp	0 - 31

F Observations in Miami, Florida indicate that the following groups of larger plants may show temporary or permanent changes following thermal discharge:

1 The sea grass Thalassia, an important habitat for invertebrates and stabilizes of the substrate.

2 Certain macro-algae

(Lurencia, Fucus, Laminaria, Macrocystis, Halimeda, and Acetabularia)

3 The phytoplankton (see Figure 7)

4 The epiphytic micro-algae

5 The benthic micro-algae

G The upper limits of thermal tolerance for two species of copepods from Chesapeake Bay were found to be near the normal temperature of the habitat during the summer. The addition of chlorine to the cooling water killed all copepods passing through the system at temperatures below the upper limits of thermal tolerance.

Dry weight of total estuarine epifauna production averaged 2.8 times greater in the discharge canal than in the intake area over a 5 year period in another study.

VI SUMMARY

The various environmental factors cannot be considered as isolated entities, organisms respond to the entire environment. Temperature criteria thus must be based on the requirements of the entire aquatic population, and on the life history requirements for different seasons of the year.

REFERENCES

1 Berger, Bernard B. Does Production of Power Pollute our Rivers? Power Engineering. March 1961.

2 Cairns,J. Jr. Effects of Increased Temperature on Aquatic Organisms Industrial Wastes. Vol. 1 (4): 150-152. 1956.

3 Clark, S.M. and Snyder, G.R. Timing and Extent of a Flow Reversal in the Lower Columbia River. Jour. Limn. of Ocean. Vol. 14. November 1969.

4 FWPCA, Water Quality Criteria Section III, Fish, Other Aquatic Life, and Wildlife. 1968.

5 FWPCA, Northwest Water Laboratory. Industrial Waste Guide on Thermal Pollution. Revised 1968.

6 FWPCA Presentations, ORSANCO Engineering Committee, Seventieth Meeting, Terrace Hilton Hotel, Cincinnati, Ohio. September 10, 1969.

7 Heibrun, L.V. Heat Death, Scientific American. pp. 70-75. April 1954.

8 Pennsylvania State of. Heated Discharges.... Their Effect on Streams Pub. No. 3, Div. San. Engr., Bur. Environmental Health, Dept. of Health. January 1962.

9 Trembley, F.J. Research Project on Effects of Condenser Discharge Water on Aquatic Life. Progress Report. Inst. of Research. Lehigh Univ. November 21, 1960.

10 Chesapeake Science. Proceedings of the 2nd Thermal Workshop of the U.S. International Biological Program. Solomons, Md. Vol. 10 (3-4). 1969.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

BIOTIC EFFECTS OF SOLIDS

I Sedimentation of rivers, lakes, estuaries and adjacent coastal water should be considered as a special case of pollution resulting from deforestation, overgrazing and faulty agricultural practices, road construction, and other land management abuses.

A Good farming practices can do a great deal to prevent silt from reaching streams and lakes.

B Road building and housing development projects, placer mining, strip mining, coal and gravel washing, and unprotected road cuts are important sources of turbidity that can be reduced with planning, good housekeeping, and regulation.

II Setteable solids include both inorganic and organic materials which may settle out rapidly, forming bottom deposits of both inorganic and organic solids.

A They may adversely affect fisheries by covering the bottom of the stream or lake with a blanket of material that destroys the bottom fauna or the spawning grounds of fish (Figure 1 from Ingram, et al).

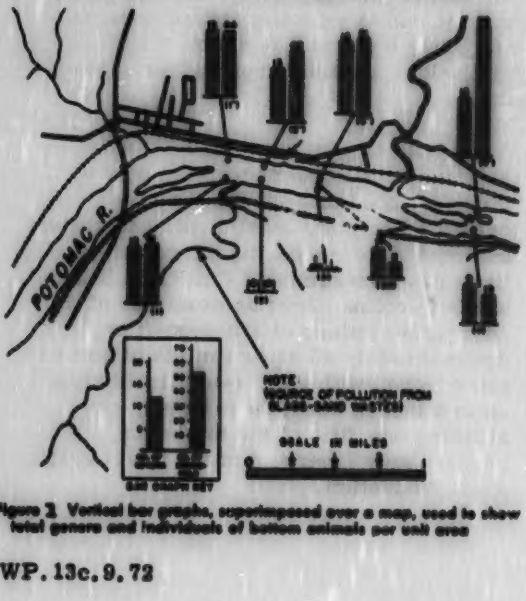


Figure 1 Vertical bar graphs, superimposed over a map, used to show total genera and individuals of bottom animals per unit area

B The organic fraction includes such setteable materials as greases, oils, tars, animal and vegetable fats, feed lot wastes, paper mill fibers, synthetic plastic fibers, sawdust, hair, greases from tanneries, and various settleable materials from city sewers. Deposits containing organic materials may deplete bottom oxygen supplies and produce hydrogen sulfide, carbon dioxide, methane, or other noxious gases.

C The inorganic components include sand, silt, and clay originating from such sources as erosion, placer mining, mine tailing wastes, strip mining, gravel washing, dusts from coal washeries, loose soils from freshly plowed farm lands, highway, and building projects.

D Some settleable solids may cause damage by mechanical action.

E The biota of streams is limited by the type of substrate.

1 A depositing substrate generally contains fewer types and may be dominated by burrowing forms.

2 An eroding substrate has a characteristic fauna of sessile attached and foraging members, such as bryozoans, stoneflies, nonburrowing mayflies, and net-spinning caddis flies.

3 The addition of solids over an originally eroding riffle substrate brings about pronounced changes in the biological community from diatoms to fish. The following are common macroinvertebrates of this new "trickling filter" community in contrast to E 2 above.

a Oligochaetes

b Alderfly larvae (Sialis)

c Midge larvae (Chironomids)

III Turbidity, color, and transparency are closely interrelated phenomena in water. They must be observed simultaneously because transparency is a function of turbidity, water color, and spectral quality of transmitted light.

A Turbidity is an expression of the optical property of a sample of water which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.

B Turbidity is caused by the presence of suspended matter such as clay, silt, finely divided organic matter, bacteria, plankton, and other microscopic organisms.

1 Algae, turbidity from silts and clays, and color of the water all affect one environmental factor of major importance in the productivity of aquatic wildlife habitat--light penetration of the water.

a Excessive turbidity reduces light penetration in the water and, therefore, reduces photosynthesis by phytoplankton organisms, attached algae, and submersed vegetation.

b The results of many of man's activities, including agriculture, industry, navigation, channelization, dredging, land modification, and eutrophication from sewage or fertilizers, often reduce light transmission to the degree that aquatic angiosperms of value to wildlife cannot grow.

c Mixed effluents from various industrial plants and domestic sewage increase the turbidity of receiving water. It is difficult to distinguish between the effect of the attenuation of light due to suspended particles and the direct effect of the particles in suspension on the growth physiology of aquatic organisms.

2 In many coastal waters, the principal cause of turbidity is the discharge of silt carried out by the principal rivers. Secchi disc readings show that the transparency of water at the mouths of large rivers during flood stage may be reduced to a few centimeters. At normal river stages, the disc may be visible at several meters below the surface.

3 Dredging of bays and tidal rivers for improvement of navigation occasionally presents serious problems. Benthic communities in the area near dredging operations may be destroyed or damaged by spoil deposition, increase in water turbidity, release of toxic substances accumulated in the mud of the polluted areas, and by changing the pattern in the dredged area.

IV PHYSICAL DAMAGES FROM SILTATION

A Silt and sediment are particularly damaging to gravel and rubble-type bottoms. The sediment fills the interstices between gravel and stones, thereby eliminating the spawning grounds of fish and the habitat of many aquatic insects and other invertebrate animals such as mollusks, crayfish, fresh water shrimp, etc.

B Accumulation of silt deposits is destructive to marine plants, not only by the associated turbidity, but by the creation of a soft, semi-liquid substratum inadequate for anchoring the roots. Back Bay, Virginia and Currituck Sound, North Carolina serve as examples of the destructive nature of silt deposition. Approximately 40 square miles of bottom are covered with soft, semi-liquid silts up to 5 inches deep; these areas, constituting one-fifth of the total area, produce only 1 percent of the total aquatic plant production.

V SILT POLLUTION INCLUDES NOT ONLY PURELY PHYSICAL EFFECTS, BUT ALSO MAY INCLUDE COMPLEX MATERIAL.

A Pollution in the estuary may be derived from contamination hundreds of miles upstream in the river basin or it may be of purely local origin. Silt plays a major role in the transport of toxicants, especially pesticides, down to the estuary.

1 Agricultural chemicals are adsorbed on silt particles. Under poor farming practices, as much as 11 tons of silt per acre per year may be washed by surface water into a drainage basin.

2 Surface mining and deforestation further accelerate the process of erosion and permit the transport of terrestrial chemical deposits to the marine environment.

B Oil that settles to the bottom of aquatic habitats can blanket large areas and destroy the plants and animals of value of waterfowl.

1 Reportedly, some oil sludges on the bottoms of aquatic habitats tend to concentrate pesticides, thus creating a double hazard to waterfowl that would pick up these contaminants in their normal feeding process.

2 Observations on storage of carcinogenic compounds found in oil-polluted water and on affected sediments are biologically significant, since they may be concentrated by commercially harvested bivalves.

C Much of the tonnage of aerially applied pesticides fails to reach the designated spray areas and the presence of 5 μg/l of DDT in presumably untreated Alaskan rivers indicates the magnitude of this facet of the pollution problem.

1 The continuous presence of 5 μg/l of DDT in the marine environment would decrease the growth of oyster populations by nearly 50 percent.

2 Atmospheric drift is also an important factor in the transport of a variety of pollutants to the aquatic environment.

3 Organochlorine compounds from sources other than pesticides applications are involved in food webs and biological magnification in remote polar environments.

D "The data on water pollution, however, are less encouraging. Among other things, they indicate that land runoff from farms and even urban land, as opposed to discharges from cities and factories, has a much greater impact on water pollution than we realized. In all types of river basins, the concentration of nutrients, which can eutrophy our lakes, is increasing. These data indicate that while we carry on our major efforts to clean up pollution from municipal and industrial sources, we must increasingly turn our attention to land runoff-of nutrients, fertilizers, pesticides, organic materials, and the soil particles that often transport the others. If we fail to do so, our expenditures for water quality will not achieve maximum improvement." Council on Environmental Quality.

REFERENCES

1 Cairns, John. Suspended Solids Standards for the Protection of Aquatic Organisms. Proc. 22, Ind. Waste Conf., Purdue Univ. Ext. Ser. 129:16. 1967.

2 FWPCA Southeast Water Lab. Role of Soils and Sediment in Water Pollution Control. Part I., Athens, Ga. 90 pp. 1968.

3 FWPCA Missouri Basin Region. Second Compendium on Animal Waste Management. USDA, Kansas City, Mo. 256 pp. 1969.

4 Hall, James D. Alsea Watershed Study (To determine the effects of logging on aquatic resources). Dept. Fish & Wildlife. Oregon State Univ. 11 pp. 1967.

5 Isom, Billy G. The Mussel Resources of the Tennessee River, Malacologia. 7(2-3):397-423. 1969.

6 Manheim, Frank T., Meade, Robert H., and Bond, Gerard C. Suspended Matter in Surface Waters of the Atlantic Continental Margin from Cape Cod to the Florida Keys. Science 167(3917):371-376. 1970.

7 Weidner, R.B, Christianson, A.G., Weibel, S.F. and Robeck, G.G. Rural Runoff as a Factor in Stream Pollution. JWPCF. 41(3):377-384.

8 Patrick, Ruth. Effect of Suspended Solids, Organic Matter and Toxic Materials on Aquatic Life in Streams. Water & Sewage Works. 115:89. 1968.

9 Council on Environmental Quality. Environmental Quality, The Third Annual Report. Aug. 1972.

This outline was prepared by Ralph M. Sinclair, Aquatic Biologist, National Training Center, OWP, EPA, Cincinnati, OH 45268.

THE CYCLING OF RADIONUCLIDES IN THE AQUATIC ENVIRONMENT

I INTRODUCTION

The desirability of plankton (tiny dispersed plants and animals) as objects of radiobiological investigation arises from a number of points:

A Their widespread occurrence in many kinds of aquatic habitats.

B Their ability to take up and to concentrate radionuclides, which may be injected, metabolized, and stored by higher organisms, including humans.

C Producing potential health hazards from ionizing radiations at the higher levels of concentration from radioactivity.

II PRELIMINARY DEFINITIONS AND CONCEPTS

A Radioecology of plankton and the food chain they begin requires a knowledge of physiology for the metabolic activities involved in the uptake of radioactive isotopes, as well as certain physical laws for the passive (dead) uptake of radionuclides, i.e., diffusion, imbibition.

B Some isotopes may be passively and instantaneously absorbed on the surface of the cells and are not taken into the cells. Cells of Carteria remove yttrium from the aquatic environment by adsorption on the surface, while the same cells take calcium and a similar substance strontium into the cells by absorption. These cells cannot, however, substitute strontium for calcium in their metabolism, because cells without calcium do not divide.

C In some cases, the concentration ratio will vary directly with the concentration of the isotope in the water, this being a linear function. In other cases, the isotope will be concentrated only to the extent that it is used. In still other cases, as phosphorus metabolism, the element may be concentrated considerably out of proportion to the available phosphorus in the aquatic environment.

D The concentration factor is the ratio of the activity of the wet weight of washed algal cells (disintegrations per minute, per gram) to the activity of the aquatic solution in which the organism was living (disintegrations per minute, per milliliter). By using concentration factors, planktonic algae may be compared for ability to concentrate radionuclides.

E A succession of organisms, beginning with those having photosynthesis (producers), and proceeding to herbivorous, omnivorous, and carnivorous organisms (consumers), each serving as a sustenance for the next, is called a food chain or cycle. Types of organisms are found at the bottom, middle, and the top of each of these chains.

III Phytoplankton (algae) take up and concentrate many kinds of isotopes, both stable and radioactive. The amount taken up of each isotope may vary with the age of the cells, the concentration of the dissolved or ionized isotope, and the pH. A rise in temperature may increase the diffusion, but could slow down the metabolism, which in turn would tend to decrease the active (respiratory) uptake of the isotope.

A Algae are noted for their ability to take up and to concentrate certain dissolved minerals from great dilutions in their environment.

1 Concentration of "essential" minerals (macronutrients), i.e., "C HOPKINS CaFe Mg NaCl."

2 Concentration of trace minerals (micronutrients) such as Cu, B, Mn, Zn, and Mo.

3 Accumulation of nonmetabolic substances

B Because of their minute sizes, phytoplankton present a great deal of surface for exchange with ions and substances in solution in water.

1 At times, algae occur in massive quantities (blooms). Under these conditions, they may selectively exhaust (take up) dissolved substances (phosphates, nitrates, and carbonates).

2 From inorganic substances are synthesized organic substances which become assimilated as protoplasm, or nonliving cell walls, etc.

3 Because they selectively take some substances out of solution, plankton have been used to decontaminate a water supply of known unwanted dissolved substances, or to reclaim valuable dissolved minerals.

4 Algae frequently concentrate substances without any known role to metabolism, i.e., cesium, rubidium, iodine, bromine, strontium, and yttrium.

IV Plants with photosynthesis are at the base (beginning) of the food chain for all aquatic life.

A In photosynthesis, plankton use radiant energy, CO_2, and H_2O to begin the elaboration of many complex organic compounds which are present in the web of aquatic life (biosphere). Radionuclides tied to these organic compounds may accumulate to concentrations dangerous to metabolism all along the food chain, depending on the selective habits of the herbivorous and predaceous animals. A "hot" fish may be radioactive from having fed on caddis fly larvae, which in turn fed on "hot" algae. The relative position in the food pyramid determined the concentration of the radioisotope.

B During periods of elaboration of large planktonic populations (blooms) peaks of radioactivity will occur near the surface with the algae. Consequently, this radioactivity may be manifested as a surface phenomenon moving down stream; however, in bodies of water with little or no current (lakes and ponds), the radioactivity may be concentrated with the algal blooms, in a stationary position.

C On degradation of the dead algal cells, the detritus formed from the algae may carry some of the radioactivity to the bottom in the form of silt making "hot" conditions for bottom-dwelling organisms.

D In a flowing stream, the specific activity will diminish along the food chain.

V METHODS OF COLLECTING INFORMATION ABOUT POSSIBLE RADIOACTIVITY OF PLANKTON AND THE FOOD CHAINS THEY COMMENCE

A Take a sample of plankton from the stream and measure its radioactivity.

B Control culture the plankton with the medium dosed with known concentrations of radioisotopes, so as to calculate the concentration factors for each radionuclide.

C Add plankton-feeding animals to an aquarium containing "hot" algae to measure the amount of radioactive uptake from the feeding of the animals on the plankton.

D Vary pH, temperature, time of exposure, age of culture and cells and note large changes in the concentration factor.

VI SOURCES OF RADIONUCLIDES

A Naturally Occurring

1 In soils, rock, etc. (uranium, radium)

2 In biota--mostly K^{40}

B Nuclear Testing

1 Air

2 Ground

3 Water and,

4 Beneath ground

C Thermonuclear Wars

D Disposal of Radionuclides

1 From power reactors

Activation of material exposed to neutron flux. Fission products

2 From widespread use of radioactive isotopes in medicine and industry

3 Nuclear powered submarines, surface ships and aircraft

4 Burial

In the oceans; in abandoned salt mines, etc., in space

VII RELATIVE DISTRIBUTION OF RADIONUCLIDES IN AQUATIC ENVIRONMENTS

A Water

B Sediment

C Biota

VIII METHODS OF BIOLOGICAL CYCLING OR RADIONUCLIDES

A Uptake and Retention

1 Active (metabolic)

2 Passive (adsorption) and simple diffusion

IX METHODS OF QUALITATING AND QUANTITATING RADIONUCLIDES FROM AQUATIC HABITATS

X CONCLUSIONS

A Food Chains

B Chemical, Physical and Biological Factors

C Need for Continuous Monitoring

D Use of Indicator Tissues and Organisms for Specific Radionuclides in Food Chains

TABLE I. UPTAKE OF CESIUM^{137} BY SPECIES OF ALGAE

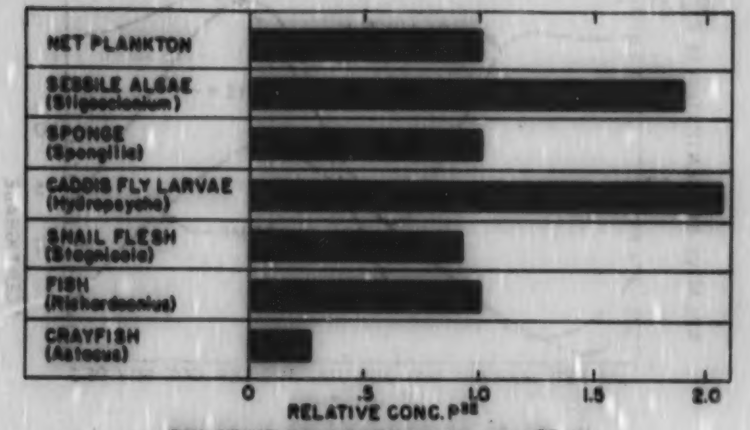
Species	ppm of k	Days after Dosing	Concentration Factors
Rhizoclonium hieroglyphicum	1	5	1530
Oedogonium vulgare	1	3	790
Spirogyra ellipsospora	1	2	341
Spirogyra communis	13	5	320
Gonium pectorale	10	2	138
Oocystis elliptica	10	10	670
Chlamydomonas sp.	8	5	52
Euglena intermedia	8	14	706
Chlorella pyrenoidosa	8	11	154
Uptake by dead (formalin-killed) cells:			
Chlorella pyrenoidosa	0.3	3	96
Euglena intermedia	0.3	3	16

TABLE II. CONCENTRATION OF CESIUM^{137} IN EUGENA

CELLS/ml		CONCENTRATION FACTORS		
BEGINNING	AT 40 HRS.	18 HRS.	40 HRS.	96 HRS.
144,000	146,100	3.4	8.2	62
86,000	113,800	3.4	11.3	58
36,000	60,000	4.6	15.4	28
21,000	68,300	5.9	17.0	26
18,000	55,500	6.4	19.4	19
1,400	27,700	5.3	11.0	16

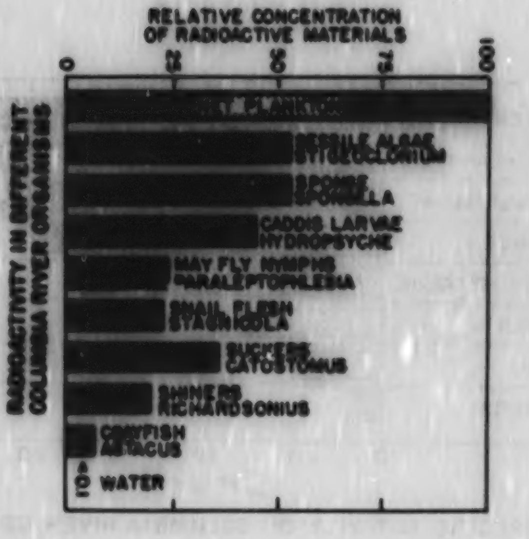
"L. G. Williams"

FIGURE 1



RELATIVE CONCENTRATION OF P^{32} IN COLUMBIA RIVER ORGANISMS

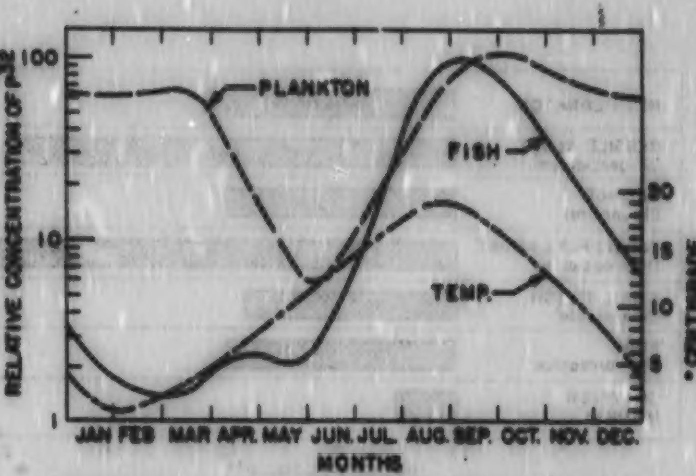
FIGURE 2



Davis and Foster, "Bioaccumulation of Radioisotopes through Aquatic Food Chains," Ecology 39: 530-535, 1958.

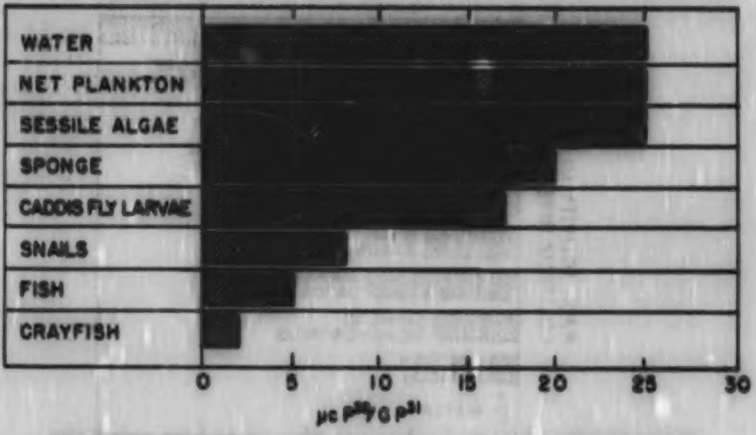
"R. F. Foster"

FIGURE 3



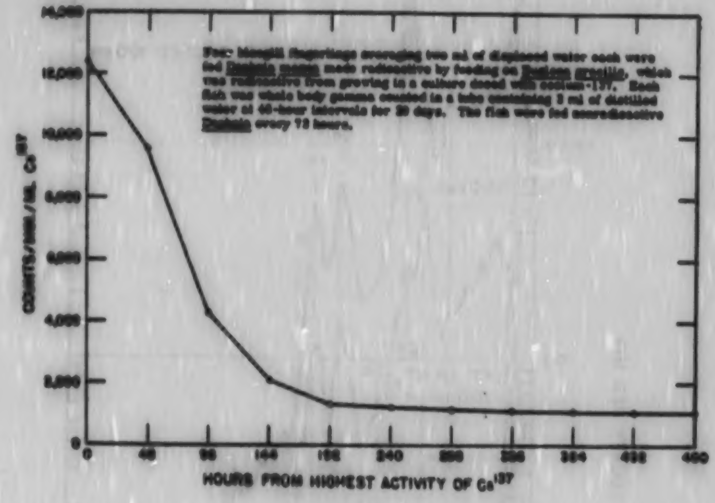
SEASONAL VARIATIONS IN THE CONCENTRATION OF P^{32} IN COLUMBIA RIVER PLANKTON AND FISH

FIGURE 4



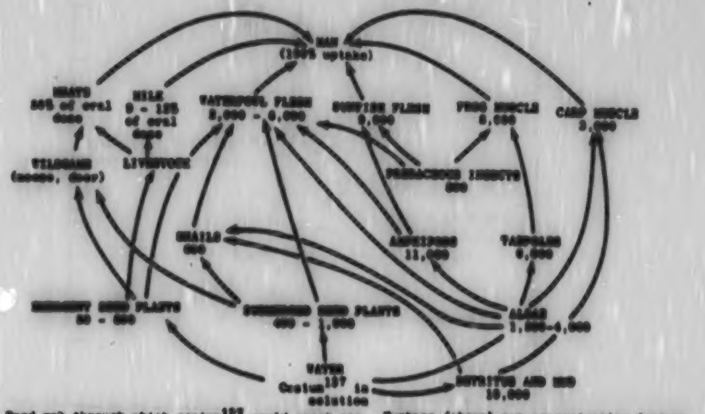
SPECIFIC ACTIVITY OF COLUMBIA RIVER ORGANISMS

"R. F. Foster"



Four bluegill fingerlings averaging two ml of displaced water each were fed *Daphnia magna* made radioactive by feeding on *Euglena gracilis*, which was radioactive from growing in a culture dosed with cesium-137. Each fish was whole body gamma counted in a tube containing 3 ml of distilled water at 48-hour intervals for 20 days. The fish were fed nonradioactive *Daphnia* every 72 hours.

FIGURE 5

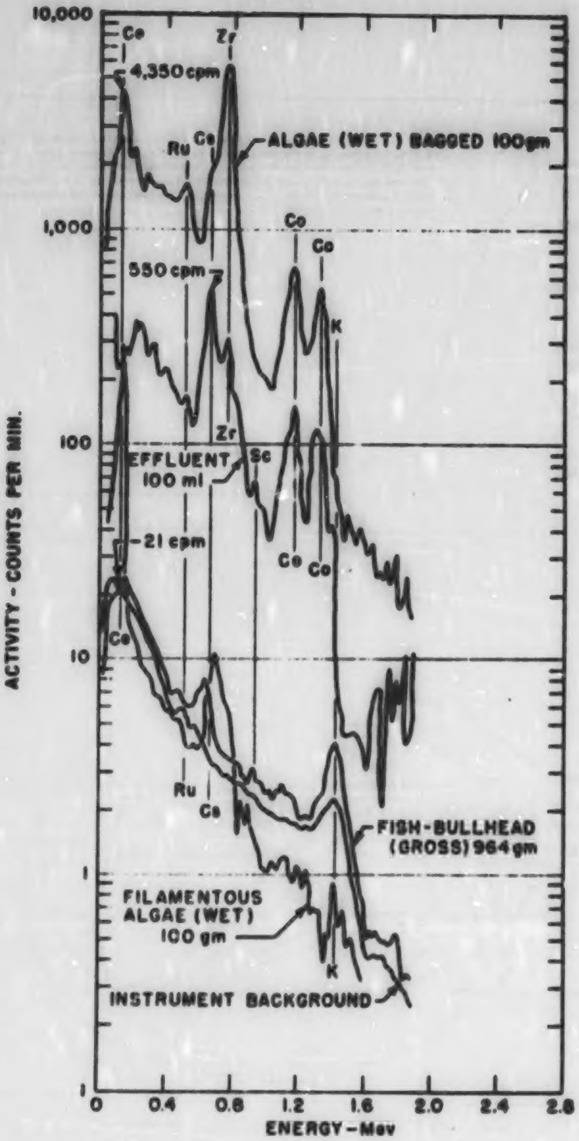

MAN
(100% uptake)
MEATS
50% of oral
dose
MILK
5 - 12%
of oral
dose
WATERFOWL FLESH
2,000 - 6,000
SUNFISH FLESH
3,000
FROG MUSCLE
3,000
CARP MUSCLE
3,000
WILDGAME
(moose, deer)
LIVESTOCK
PREDACEOUS INSECTS
800
SNAILS
600
AMPHIPODS
11,000
TADPOLES
9,000
EMERGENT SEED PLANTS
50 - 500
SUBMERGED SEED PLANTS
400 - 1,000
ALGAE
1,000-4,000
WATER
$Cesium^{137}$ in
solution
DETRITUS AND MUD
10,000

Food web through which $cesium^{137}$ could reach man. Numbers (above) are concentration factors.

Concentration factors: $\frac{Cs^{137} \text{ concentration in organism}}{Cs^{137} \text{ concentration in water}}$

"L. G. Williams, 1960"

FIGURE 7



Uptake of radionuclides from the Mohawk River, New York, at 17°C. in September, 1959, after 72 hours by *Pithophora* in perforated polyethylene bags, compared with activity of fish (bullhead) and an indigenous filamentous green alga, *Cladophora*, taken from the river. Uptake of these radionuclides by *Pithophora* was nonmetabolic, since the alga had been killed with chlorine.

"L. G. Williams"

FIGURE 8

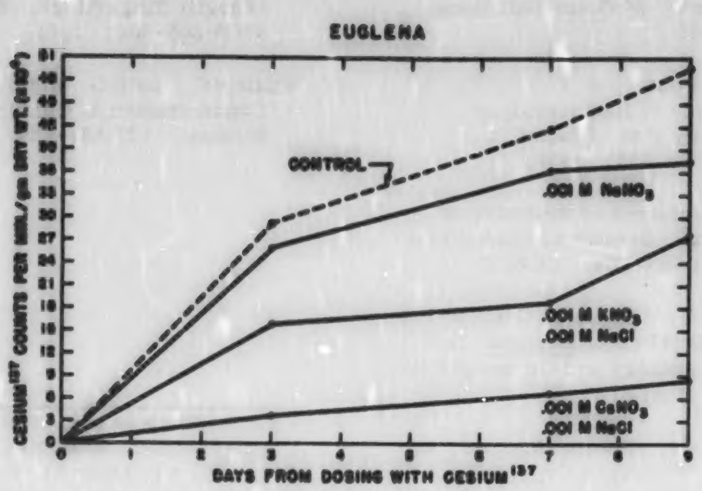
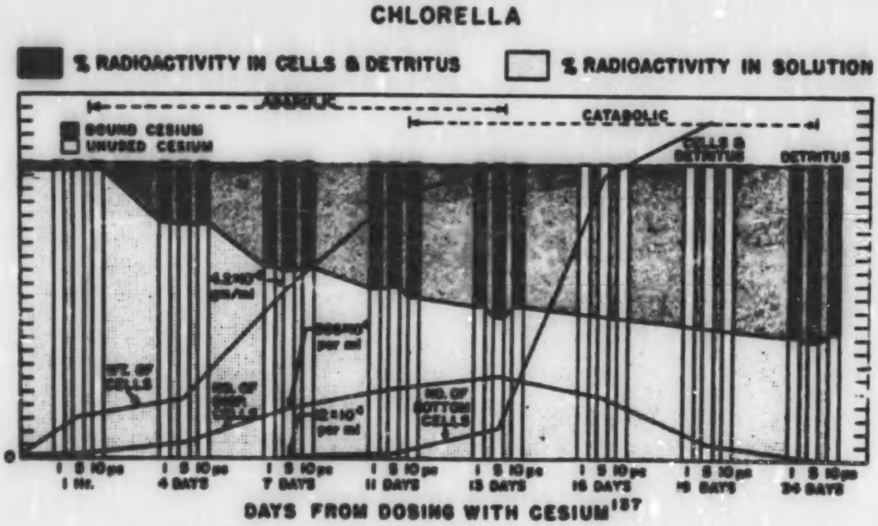


FIGURE 9



"L. G. Williams"

REFERENCES

1 Comar, C.L. Radioisotopes in Biology and Agriculture. McGraw-Hill Book Company. 1955.

2 Davis, J.J. and Foster, R.F. Bioaccumulation of Radioisotopes Through Aquatic Food Chains. Ecology. 39:530-535. 1958.

3 Straub, C.P. Problems of Radioactive Waste Disposal. Sewage and Industrial Wastes. 28(6):787-794. 1956.

4 Williams, Louis G. Uptake of Cesium-137 by Cells and Detritus of Euglena and Chlorella. Limnology and Oceanography. 5(3):301-311. 1960.

5 Williams, Louis G., Howell, Michael, and Straub, C.P. Packaged Organic Materials as Monitoring Tools for Radionuclides. Science. 132(3439):1554-1555. 1960.

6 Williams, Louis G. and Pickering, Quentin. Direct and Food-Chain Uptake of Cesium-137 and Strontium-85 in Bluegill Fingerlings. Ecology. 42(1):205-206. 1961.

7 Williams, Louis G. and Swanson, H.D. Concentration of Cesium-137 by Algae. Science. 127:187-188. 1958.

This outline was prepared by W. A. Brungs, Jr., Former Aquatic Biologist, Fish Toxicology Activities and Louis G. Williams, Former Biologist, Research and Development, Cincinnati Water Research Laboratory, FWQA, SEC.

BACTERIA AND PROTOZOA AS TOXICOLOGICAL INDICATORS

I INTRODUCTION

The WINOGRADSKY COLUMN is an excellent simple classroom experiment as well as a miniature ecosystem which yields a variety of photosynthetic and other protista especially the bacterial forms important in photosynthesis research. These photosynthetic bacteria, as pointed out by Dr. Hutner, are ubiquitous in wet soils and natural waters but ordinarily escape notice.

II PREPARING THE COLUMN

The materials needed are simple laboratory items.

A The inoculum (a black sludge) may be easily obtained from a local sewage treatment plant or the bottom of a pond or lake. Because the USPHS document, containing Dr. Hutner's paper, is out of print it is reproduced here.

B Directions for preparing the column and other useful information are given in that paper.

C Dr. Hutner's bibliography should be sufficient for those who wish more information.

III ECOSYSTEM DEVELOPMENT

A Factors such as the substrate used, the inoculum, the overlying supernatant water, and laboratory conditions as temperature and light, will all influence the particular type of biota forming successive layers or zones. The accompanying figure is therefore generalized and is not intended to be absolute.

B Some representative forms are listed for general information. The numbers correspond to those on the figure.

1 Inorganic substrate on toweling.

2 Green photosynthetic bacteria Microchloris, Chlorobium, and Chlorochromatum; methane bacteria; and SO_4 reducers.

3 Photosynthetic purple sulfur bacteria. Thiopedia and Thiosarcina.

4 Filamentous sulfur bacteria, Beggiatoa.

5 Non-sulfur photosynthetic bacteria, Rhodopseudomonas.

6 Blue-green algae, Schizothrix and Oscillatoria.

7 Diatoms, Nitzschia.

8 Coccoid green algae, Ankistrodesmus; and flagellate greens, Chlamydomonas; similar to a stabilization pond flora.

9 Filamentous green algae, Ulothrix.

C Besides the photosynthetic bacteria and other protista there will be a variety of protozoa found in the aerobic levels (app. 6 through 9). Many of these protozoans are typical fauna of activated sludge.

D The possibilities are endless for further experimentation. These ecosystems are also convenient and inexpensive sources for protozoa and other protista for class and laboratory instruction.

IV MICRO AQUARIA

A Fenchel describes a micro aquarium (1.5 × 5 cm) which may be observed under a compound microscope. (Figure 2)

B The development of communities of organisms is quite similar to the Winogradsky Column. (Figure 3)

C The basic media consists of one liter of natural water 10 g $CaSO_4$, 1 g glucose, 1 g of peptone, autoclaved and stored at 5°C.

D Before use agar is boiled with the media. While hot, the media is introduced into one end of the "micro aquarium" with a pipet. After the agar congeals, natural water samples are added. During incubation and when not being observed, the micro aquaria are kept in a moist chamber.

E Although Fenchel used a seawater medium and inoculum, freshwater sources would be equally useful.

F In these microaquaria simple ecosystems develop when they are kept in complete darkness. Complex photosynthetic communities develop when they are illuminated. A natural ecosystem is figured by Fenchel (Figure 4), and a related food web is shown in Figure 5 (both from Fenchel).

G Microaquaria Using Plastic Petri Dishes

Sessile ciliates have been successfully collected, cultured, and used for bioassay using the same petri dish (membrane filter type, with tight fitting lids).

H Microaquaria using silicone cement rings which allow diffusion of gases through the silicone cultures will thereby remain active indefinitely.

ADDITIONAL REFERENCES

1 Hutner, S.H. Protozoa as Toxicological Tools. The Jour. of Protozoology. 11(1):1-6. 1964.

2 Spoon, D.M. and Burbanck, W.D. A New Method for Collecting Sessile Ciliates in Plastic Petri Dishes with Tight-Fitting Lids. Jour. of Protozoology 14(4):735-739. 1967.

3 Burbanck, W.D. and Spoon, D.M. The Use of Sessile Ciliates Collected in Plastic Petri Dishes for Rapid Assessment of Water Pollution. Jour. of Protozoology. 14(4):739-744. 1967.

4 Curds, C.R. and Cockburn, A. Protozoa in Biological Sewage Treatment Processes. I. A Survey of the Protozoan Fauna of British Percolating Filters and Activated Sludge Plants. Water Research 4:225-236. 1970.

5 Curds, C.R. and Cockburn, A. II. Protozoa as Indicators in the Activated Sludge Process. Water Research 4:237-249. 1970.

6 Curds, C.R. An Illustrated Key to the British Freshwater Ciliated Protozoa Commonly Found in Activated Sludge. Water Poll. Research Laboratory. Stevenage, England. 90 pp.

7 Fenchel, Tom. The Ecology of Marine Microbenthos. IV. Structure and Function of the Benthic Ecosystem, its Chemical and Physical Factors and the Microfauna Communities with Special Reference to the Ciliated Protozoa. Ophelia 6:1-182. 1969.

8 Hutner, S.H. Botanical Gardens and Horizons in Algal Research. In Challenge for Survival. Pierre Dansereau ed. Columbia University Press. 1970.

9 Hutner, S.H.; Baker, H.; and Cox, D. Nutrition and Metabolism in Protozoa. Chapter in Biology of Nutrition. pp. 85-177. edited by R.N. Fiennes. Pergamon Press. 1972.

10 Hutner, S.H. The Urban Botanical Garden: An Academic Wildlife Preserve. Garden Journ. 19(2):37-40. 1969.

This outline was prepared by Ralph M. Sinclair, Aquatic Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45226.

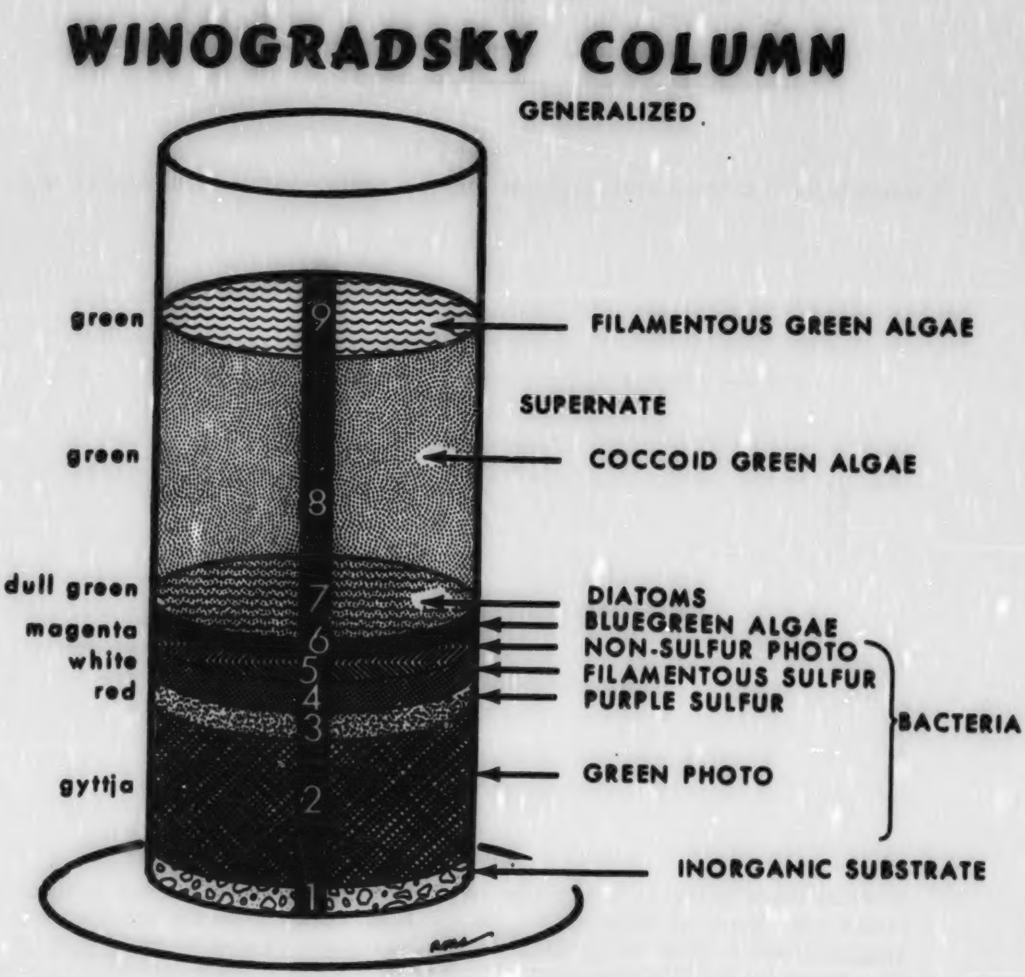

WINOGRADSKY COLUMN
GENERALIZED
green
FILAMENTOUS GREEN ALGAE
SUPERNATE
green
COCCOID GREEN ALGAE
dull green
DIATOMS
magenta
BLUEGREEN ALGAE
white
NON-SULFUR PHOTO
red
FILAMENTOUS SULFUR
PURPLE SULFUR
BACTERIA
gyttja
GREEN PHOTO
INORGANIC SUBSTRATE
9
8
7
6
5
4
3
2
1

FIGURE 1

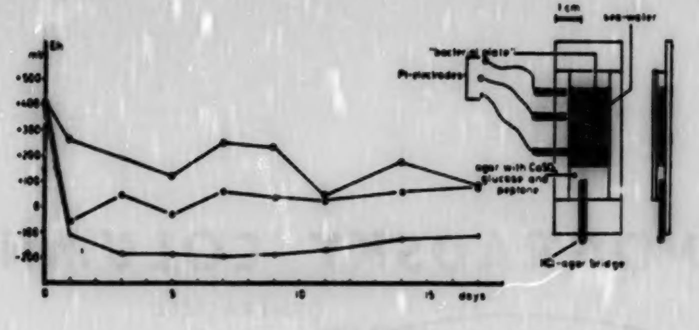


FIGURE 2

A micro aquarium fitted with electrodes and the redox conditions through 17 days.

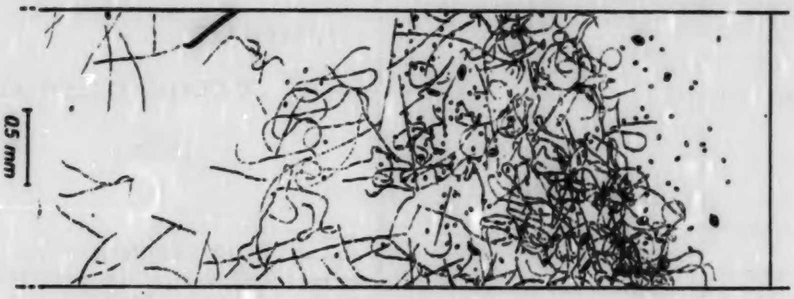


FIGURE 3

Drawing made by tracing a micrograph of the bacterial plate in a micro aquarium (same experiment as shown on Figure 2.) Most conspicuous are the filaments of Beggiatoa and the ciliates Cyclidium citrullus, Euplotes elegans and Holosticha sp. Below the Oscillatoria filament (lower left) a Plagiopogon loricatus is seen. Bacteria (except Beggiatoa) are not shown.

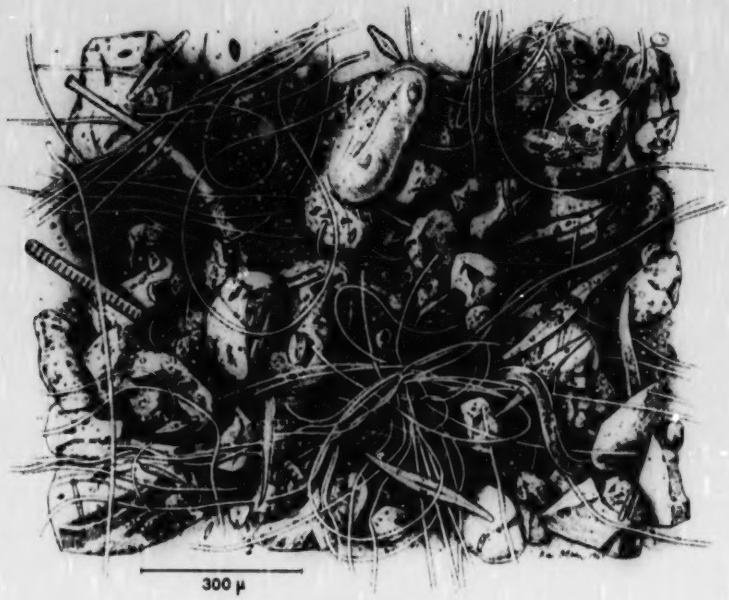


FIGURE 4

The microflora and fauna in the surface of the Beggiatoa patches. (Oscillatoria, Beggiatoa, Thiovolum, diatoms, euglenoids, nematode, Tracheloraphis sp., Frontonia marina, Diophrys scutum, Trochiloides recta).

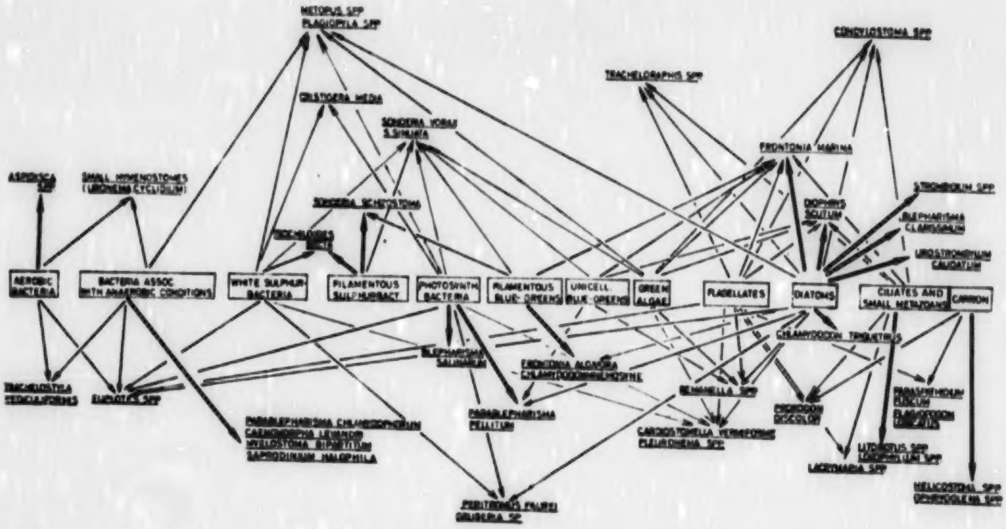

METOPUS SPP
PLAGIOPYLA SPP
CRISTIGERA MEDIA
SONDERIA VORAX
S. SINUATA
CONDYLOSTOMA SPP
TRACHELORAPHIS SPP
FRONTONIA MARINA
ASPIDISCA SPP
SMALL HYMENOSTOMES (URONEMA CYCLIDIUM)
SONDERIA SCHIZOSTOMA
DIOPHRYS SCUTUM
STROMBIDIUM SPP
BLEPHARISMA CLAPAREDEI
UROSTRONGYLUM CAUDATUM
AEROBIC BACTERIA
BACTERIA ASSOC WITH ANAEROBIC CONDITIONS
WHITE SULPHUR BACTERIA
FILAMENTOUS SULPHURBACT
PHOTOSYNTH BACTERIA
FILAMENTOUS BLUE-GREENS
UNICELL BLUE-GREENS
GREEN ALGAE
FLAGELLATES
DIATOMS
CILIATES AND SMALL METAZOANS
CARRION
CHLAMYDODON TRIQUETRUS
BLEPHARISMA SALINARUM
TRACHELOSTYLA PEDICULIFORMIS
EUPLOTES SPP
PARABLEPHARISMA CHLAMYDOPHORUM
CAENOMORPHA LEVANDERI
MYELOSTOMA BIPARTITUM
SAPRODINIUM HALOPHILA
PARABLEPHARISMA PELLITUM
PERITROMUS FAURPI
GRUBERIA SP
REMANELLA SPP
CARDIOSTOMELLA VERMIFORME
PLEURONEMA SPP
PRORODON DISCOLOR
LITONOTUS SPP
LOXOPHYLLUM SPP
LACRYMARIA SPP
HELICOSTOMA SPP
OPHRYOGLENA SPP

FIGURE 5

The food relationships of the most common ciliates in "estuarine" sediments and in sulphureta.

ENVIRONMENTAL REQUIREMENTS OF FRESH-WATER INVERTEBRATES

L. A. Chambers, *Chairman

Bacteria-Protozoa as Toxicological Indicators in Purifying Water

S. H. Hutner, Herman Baker, S. Aaronson and A. C. Zahalsky+

There is a cynical adage that all travelers become entomologists. But, now with DDT and detergents, travelers and stay-at-homes alike are becoming toxicologists. We have an immediate interest in pollution problems: Our laboratory receives, like the East River and adjoining United Nations buildings, a generous sootfall from a nearby power plant. Also, we have seen a superb fishing ground, Jamaica Bay in New York City, become a sewer. (Jamaica Bay is, however, being restored to its pristine cleanliness--but not the U.N. area.) We take our theme nevertheless not from aesthetics but from statements by Berger (1961): (1) It is an expensive, time-consuming project"...to predict with confidence a new waste's probable impact on certain important downstream water uses." And (2) "The toxicological phase of the study is perhaps its most perplexing aspect. The specialized services and cost necessary for determining the effect of repeated exposure to low concentrations of the waste for long periods of time would inevitably place this job out of reach of most public agencies. Equally discouraging, perhaps, is the probability that the toxicological study may take as long as two years."

As described here, advances in microbiology offer hopes of lightening this burden. The first question is: What kind of microcosm will serve for toxicological surveys, especially for predicting the poisoning of biological means of waste disposal? The second is: Can the protozoa of this microcosm predict toxicity to higher animals?

The food-chain pyramids of sewage plants and polluted waters have been adequately described (Hynes, 1960; Hawkes, 1960). A problem treated here is how to scale those microcosms to experimentally manipulable microcosms yielding dependable predictions for the behavior of sewage-plant microcosms.

THE WINOGRADSKY COLUMN AS SOURCE OF INOCULA FOR MINERALIZATIONS AND AS TOXICOLOGICAL INDICATOR SYSTEM

Total toxicity depends on intrinsic toxicity-persistence relationship. Techniques for testing the degradability of organic compounds--and so their persistence in soil and water--are still haphazard. The enrichment culture technique, in which one seeks microbes that use the compound in question as sole substrate--hence degrades it and even "mineralizes" it--was developed by the Dutch school of microbiologists. Enrichment cultures are used routinely by biochemists wishing to work out the microbial catabolic metabolic pathway for a compound of biochemical interest. Since the compounds dealt with by biochemists are of biological origin, microbial degradability can be assumed. Still, finding a microbe to degrade a rare biochemical is not always easy: Dubos, in a classical hunt for a microbe able to live off the capsules of pneumococci, found the bacterium only after a long search which ended in a cranberry bog. Such difficulty in finding microbes that degrade rare biochemicals implies an even greater difficulty in finding microbes that degrade many products of the synthetic

---

*Director, Allan Hancock Foundation and Head, Bio. Dept., Univ. South. Calif.

+Haskins Laboratories, 305 E. 43rd St., New York 17, N.Y.; and Seton Hall College of Medicine and Dentistry, Jersey City, N.J. Pharmacological work from Haskins Laboratories discussed here was assisted by grant R6-9103, Div. of General Medical Sciences of the National Institutes of Health. Paper presented by S. H. Hutner.

Preceding page blank

organic chemicals industry, since such compounds may embody biochemically rare or biochemically nonexistent linkages. Intimations of the importance of inoculum abound in the literature, e.g., Ross and Sheppard (1956) could not at first obtain phenol oxidizers from ordinary inocula (presumably soil and water), but manure and a trickling filter from a chemical plant proved abundant sources of active bacteria. One wonders how extensive a study underlies the statement quoted by Alexander (1961) that "soils treated with 2,4, 5-T (trichlorophenoxy-acetic acid) still retain the pesticide long after all vestiges of toxicity due to equivalent quantities of 2, 4-D have disappeared."

What then is a reasonable inoculum for testing a compound's susceptibility to microbial attack? The size range is wide: from the traditional crumb or gram of soil or mud to the scow-load of activated sludge contributed by New York City to inaugurate the Yonkers sewage-disposal plant. We suggest that to strike a practical mean in getting a profile of soil, mud, or sludge to be used as inoculum the uses of the Winogradsky column should be explored. Directions for Winogradsky column and bacteriological enrichments have been detailed (Hutner, 1962) and so only an outline is given here. The column is prepared by putting a paste of shredded paper, $CaCO_3$, and $CaSO_4$ at the bottom of a hydrometer jar, filling the jar with mud smelling H_2S, covering with a shallow layer of water, and illuminating from the side with an incandescent lamp. In 2 or 3 weeks sharp zones appear: a green-and-black anaerobic zone at the bottom (a mixture of green photosynthetic bacteria along with SO_4-reducers, methane producers, and the like); over that a red zone (predominantly photosynthetic bacteria); above this garnet or magenta spots or zone (predominantly non-sulfur photosynthetic bacteria); above this a layer rich in blue-green algae (the transition to the aerobic zones); above this aerobic bacteria along with green algae, diatoms, other algae, and an assortment of protozoa. This makes an excellent simple classroom experiment to demonstrate the different kinds of photosynthetic organisms, especially the bacterial forms that are important in photosynthesis research and that ordinarily escape notice yet are ubiquitous in wet soils and natural waters.

As pointed out by our colleague, Dr. L. Provasoli (1961), the heterotrophic capacities of algae are very imperfectly known. This is underscored by recent studies of the green flagellate *Chlamydomonas mundana* as a dominant in sewage lagoons in the Imperial Valley of California (Eppley and MaciasR, 1962); other than that it prefers acetate among the few substrates tried; its heterotrophic capacities are unknown. More unexpectedly, some strains of the photosynthetic bacterium *Rhodopseudomonas palustris* use benzoic acid anaerobically as the reductant in photosynthesis (Scher, Scher, and Hutner, 1962) narrowing the gap between the photosynthetic pseudomonads and the ubiquitous pseudomonads so often represented among bacteria attacking resistant substrates (e.g., hydrocarbons) as well as highly vulnerable substrates. For the widely studied, strongly heterotrophic photosynthetic flagellates *Euglena gracilis* and *E. viridis*, common in sewage, no specific enrichment procedure is known, meaning that their ecological niches are unknown but laboratory data provide hints.

The increasing use of oxidation ponds would in any case urge a greater use of scaled-down ecological systems in which development of none of the photosynthesizers present in the original inoculum was suppressed. Conceivably, some of the rare microbes attacking rare substrates--and such microbes are likely to represent a source of degraders of resistant non-biochemicals--are specialists in attacking products of photosynthetic organisms.

Traditionally, the inoculum for a Winogradsky column is a marine or brackish mud (as New Yorkers we would be partial to mud from flats of the Harlem River). Little is known about the effectiveness as inocula of freshwater muds or water-logged soils. It would be valuable to know how complete a column could develop from material from a trickling filter or an activated-sludge plant. A practical issue is: Might the poisoning of a sewage-oxidation system be paralleled by the poisoning of a Winogradsky column, where the poison was mixed with the inoculum for the column? Might the variously colored photosynthetic zones of the column and the aerobic population on top provide sensitive indicators for the performance of a sewage-oxidation system subjected to chemical wastes?

If a particular compound mixed with inoculation mud or sludge suppressed development of the full Winogradsky pattern, one might assume that the compound at the test concentration was poisonous and persistent. Biological destruction of such poisons, if at all possible, might demand a long hunt for suitable microorganisms, then buildup of the culture to a practical scale. This might best be done with illuminated shake or aerated cultures, with the inocula coming from a variety of environments. Optimism that microbes can be found capable of breaking almost all the linkages of organic chemistry is fostered by the study of antibiotics, which include a wealth of previously "unphysiological" linkages--azo compounds, oximes, N-oxides, aliphatic and aromatic nitro and halogen compounds, and strange heterocyclic ring systems. Some natural heterocycles, e.g., pulcherriminic acid and 2-n-nonyl-4-hydroxyquinoline, have a disquieting resemblance to the potent carcinogen 4-nitroquinoline N-oxide.

PROTOZOA AS TOXICOLOGICAL TOOLS

A difficult problem is one mentioned earlier: persistence joined with low-grade toxicity to higher animals. Recent developments in the use of protozoa as pharmacological tools show that protozoa can serve as sensitive detectors of metabolic lesions ("side actions"?) of a wide assortment of "safe" drugs. The list includes the "anticholesterol" triparanol (MER/29). Triparanol toxicity manifested itself with several protozoa, including *Ochromonas danica* (Aaronson et al., 1962) and *Tetrahymena* (Holz et al., 1962). Triparanol was not acting simply as an anticholesterol for its obvious toxicity to protozoa was annulled by fatty acids as well as by sterols. The connection between the protozoan results and the "side actions" of triparanol--baldness, impotence, and cataracts--are of course unclear, but protozoal toxicity might serve as an initial warning that it might not be as harmless as assumed from short-term experiments with higher animals.

The anticonvulsant primidone provides a clear indication of how protozoa can be used to pinpoint the location of a metabolic lesion. Primidone had been known to cause folic acid-responsive anemias. It is therefore easy to find that with joint use of a thymine-dependent *Escherichia coli* and the flagellate *Crithidia fasciculata* reversal of growth inhibition by folic acid and related compounds permitted the charting of interferences with the interconnected folic acid, biopterin, and DNA function (Baker et al., 1962), which amply accounted for the megaloblastic anemias. *Lactobacillus casei*, a bacterium much used in chemotherapeutic research, was unaffected by the drug.

In another instance, where the mode of action of the drug in higher animals was unknown, growth inhibition property of the anticancer compound 1-aminocyclopentane-1-carboxylic acid was reversed for *Ochromonas danica* by L-alanine and glycine, as was the inhibition property of 1-amino-3-methyl-cyclohexane-1-carboxylic acid by L-leucine (Aaronson and Bensky, 1962).

Growth inhibition of *Euglena* by the potent carcinogen 4-nitroquinoline N-oxide was annulled by a combination of tryptophan, tyrosine, nicotinic acid, phenylalanine, uracil (Zahalsky et al., 1962) and, in more recent experiments, the vitamin K relative phthiocol. These N-oxides are of interest because of recent work indicating that perhaps the main way in which the body converts such compounds as the amino hydrocarbons to the actual carcinogens may be by an initial hydroxylation of the nitrogen, e.g., work by Miller et al., (1961). Whether the peroxides in photo-chemical smogs of the Los Angeles type act on hydrocarbons to produce carcinogenic N-oxides is entirely unknown. Leighton (1961) lists an array of peroxy reactions produced by sunlight in polluted air.

Our aforementioned experience with primidone, a ketonic heterocycle, led us to test the sedative thalidomide. It was toxic for *Ochromonas danica O. malhemensis* and *Tetrahymena pyriformis*; this toxicity was annulled by nicotinic acid (or nicotinamide) or vitamin K (menadione) (Frank et al, 1963). We do not know whether a similar protection could have been conferred on human embryos or polyneuritis in the adult.

Many widely used herbicides of the dinitrophenol type are powerful poisons for higher animals. We do not know how sensitive protozoa would be for detecting their persistence. However, since somewhat similar thyro-active compounds can be sensitively detected by their exaggeration of the B_{12} requirement of *Ochromonas malhamensis* (Baker et al, 1961), this flagellate might be a useful test object for dinitrophenols and congeners.

The *Paramecium* (and perhaps too the *Tetrahymena*) test for polynuclear benzenoid carcinogens has remarkable sensitivity and specificity (Epstein and Burroughs, 1962; Hull, 1962). This test depends on the carcinogen-sensitized photodynamic destruction of paramecia by ultraviolet light. This test is approaching practicality for air, and there is no reason to suppose it cannot be applied to benzene extracts of foodstuffs and water.

CONCLUSIONS

We have suggested here new procedures for examining the intrinsic toxicity-persistence relationship, using as test organisms the protists represented conspicuously in a Winogradsky column. The new field of micro-toxicology is virtually undeveloped. The urgent need for detecting chronic, low-grade toxicities is evident from many sides. This is not the place for a detailed discussion of the medical implications of this area of research, but it should be emphasized that chronic toxicities and carcinogenesis are related. Conversely, Umezawa (1961) has remarked that most antitumor substances have chronic toxicities and that elaborate testing procedures for toxicity are required to fix the daily tolerable dose; apparently this problem is a central theme in medical as well as pollution research. Inhibition of growth of an array of protozoa is now in practical use as a means of detecting anticancer substances in antibiotic beers (Johnson et al, 1962). Since the embryos appear to lack the detoxication mechanisms of the adult animal (Brodie, 1962), toxicity for protozoa (which presumably lack these detoxication mechanisms) should be compared with that for the embryo, not the adult, as emphasized by the thalidomide disaster.

There are further limitations on the use of microbes as detectors of toxicity. High-molecular toxins seem inert to microbes, and antihormones (with the exception of anti-thyroid compounds) are generally inert. The main usefulness of microbial indices of toxicity would appear, then, to be for detecting low-molecular poisons acting on cellular targets rather than on cell systems and organs. These are precisely the poisons likely to put out of business a pollution-control installation primarily dependent on microbial activity.

REFERENCES

Aaronson, S. and Bensky, B. 1962. Protozoological studies of the cellular action of drugs. I. Effect of 1-aminocyclopentane-1-carboxylic acid and 1-amino-3-methylcyclo-hexanel-1-carboxylic acid on the phytoflagellate *Ochromonas danica*. Biochem. Pharmacol. 11:983-6.

Aaronson, S., Bensky, B., Shifrine, M. and Baker, H. 1962. Effect of hypocholesteremic agents on protozoa. Proc. Soc. Exptl. Biol. Med. 109:130-2.

Alexander, M. 1961. "Introduction to Soil Microbiology", John Wiley & Sons, N.Y. (see p. 240).

Baker, H., Frank, O., Hutner, S.H., Aaronson, S., Ziffer, H. and Sobotka, H. 1962. Lesions in folic acid metabolism induced by primidone. Experientia 18:224-6.

Baker, H., Frank, O., Pasher, I., Ziffer, H., Hutner, S.H. and Sobotka, H. 1961. Growth inhibition of microorganisms by thyroid hormones. Proc. Soc. Exptl. Bio. Med. 107:965-8.

Berger, B.B. 1961. Research needs in water quality conservation. In "Algae and Metropolitan Wastes", Trans. 1960 Seminar, Robt. A. Taft Sanitary Eng. Center, Cincinnati 26, Ohio, p. 156-9.

Brodie, B.D. 1962. Drug metabolism-subcellular mechanisms. In "Enzymes and Drug Action", J. L. Mongar and A.V.S. de Reuck, eds., Ciba Foundation Symposium, J. & A. Churchill Ltd., p. 317-40.

Eppley, R.W. and MaciasR, F.M. 1962. Rapid growth of sewage lagoon Chlamydomonas with acetate. Physiol. Plantarum 15:72-9.

Epstein, S.S. and Burroughs, M. 1962. Some factors influencing the photodynamic response of *Paramecium caudatum* to 3,4-benzypyrene. Nature 193:337-8.

Frank, O., Baker, H., Ziffer, H., Aaronson, S., and Hutner, S.H. 1963. Metabolic deficiencies in protozoa induced by Thalidomide. Science 139:110-1.

Hawkes, H.A. 1960. Ecology of activated sludge and bacteria beds. In "Waste Treatment", P.C.G. Isaac, ed., Pergamon, N.Y., Oxford, etc., p. 52-97; discussion p. 97-8.

Holz, G.G., Jr., Erwin, J., Rosenblum, N. and Aaronson, S. 1962. Triparanol inhibition of *Tetrahymena* and its prevention of lipids. Arch. Biochem. Biophys. 98:312-22.

Hull, R.W. 1962. Using the "Paramecium assay" to screen carcinogenic hydrocarbons. J. Protozool. (Suppl) 9:18.

Hutner, S.H. 1962. Nutrition of protists. In "This is Life: Essays in Modern Biology", W.H. Johnson and W.C. Steere, eds., Holt, Rinehart and Winston, N.Y. P. 109-37.

Hynes, H.B.N. 1960. The Biology of Polluted Waters. Liverpool Univ. Press.

Johnson, I.S., Simpson, P.J. and Cline, J.C. 1962. Comparative studies with chemotherapeutic agents in biologically diverse in vitro cell systems. Cancer Research 22:617-26.

Leighton, P.A. 1961. "Photochemistry of Air Pollution", Academic Press, N.Y.

Miller, J.A., Wyatt, C.S., Miller, E.C. and Hartman, H.A. 1961. The N-hydroxylation of 4-acetylaminobiphenyl by the rat and dog and the strong carcinogenicity of N-hydroxy-4-acetylaminobiphenyl in the rat. Cancer Research 21:1465-73.

Provasoli, L. 1961. Micronutrients and heterotrophy as possible factors in bloom production in natural waters. In "Algae and Metropolitan Wastes", Trans. 1960 Seminar, Robt. A. Taft Sanitary Eng. Center, Cincinnati 26, Ohio, p. 48-56.

Ross, W.K. and Sheppard, A.A. 1956. Biological oxidation of petroleum phenolic wastewaters. In "Biological Treatment of Sewage and Industrial Wastes", J. McCabe and W.W. Eckenfelder, Jr., eds., 1:370-8. Reinhold Publ. Co., N.Y.

Scher, S., Scher, B. and Hutner, S.H. 1963. Notes on the natural history of *Rhodopseudomonas palustris*. In "Symposium on Marine Microbiology", ed. C.H. Oppenheimer, C.C. Thomas, Springfield, Ill., p. 580-7.

Umezawa, M. 1961. Test methods for antitumor substances. Sci. Repts. Ist. Super. Sanita 1:427-38

Zahalsky, A.C., Keane, K., Hutner, S.H., Kittrell, M. and Amsterdam, D. 1962. Protozoan response to anticarcinogenic heterocyclic N-oxides: toxicity and temporary bleaching. J. Protozool. (Suppl.) 9:12.

"Biological Problems in Water Pollution" (Third Seminar 1962) U.S. Public Health Service Pub. No. 999-WP-25, Cincinnati, OH . 1965.

PATHOGENIC MICROORGANISMS IN WATER AND SEWAGE

I GENERAL CONSIDERATIONS

A With few exceptions, all waterborne pathogens parasitic in way of life.

B With few exceptions, pathogens arise from fecal pollution directly or indirectly through sewage or sewage effluent.

C Water mechanical medium for most pathogens, a few requiring aquatic forms of life as intermediate hosts. Vibrio El Tor and V. comma, causative agent of chlora believed capable of multiplying in water under favorable conditions.

D Diseases which waterborne pathogens cause susceptible to environmental control, hence, they serve as indices of sanitary conditions of areas or countries.

E Swimming associated amoeba Meningoencephalitis apparently not connected with sewage pollution.

F Leptospirosis associated with pollution of water by agricultural land runoffs and the like.

G Comprising viruses, bacteria, protozoa, and worms.

II VIRUSES

A Enteric viruses most important, esp. virus of infectious hepatitis.

B Enteroviruses (Polio, Coxsackie, and Echo) found in domestic sewage and sewage effluent whenever and wherever examined - about 10% of children age 14 and under shedding virus in this country, higher percentages in many other countries; only two polio outbreaks blamed on water.

C Adenovirus and reovirus also discharged in feces, not so important because of respiratory disease it causes; reovirus has been reported to be involved in infant dirrhea and discharged in the feces.

D Important matters related to virus in water:

1 Concentration in sewage efficiency of treatment to remove viruses.

2 Concentration and survival in water.

3 Removal and destruction by water treatment processes.

4 Contamination of shellfish by enteric viruses in polluted shellfish growing areas.

III BACTERIA

A Waterborne bacterial diseases have declined to very low incidence in this country - only a few small outbreaks in recent years (Salmonella and Shigella). Food-bourne infections a different matter.

B Cholera confined to endemic areas of the world.

C Pathogenic E. coli could be an etiologic agent of (infantile) diarrheal disease.

D Cl. botulinum growing in bottom mud of lakes may be a health problem related to fish consumption in causing botulism.

E Mycobacterium balnie growing in mineral springs and swimming pools fed with such water and causing swimmer's elbow; keeping a watching eye on new pathogenic bacteria in water.

F Leptospiral causing enteric as well as non enteric fibrile illness.

G Important matters related to pathogenic bacteria in water:

1 Concentration in sewage, sewage effluent, and water

2 Survival in water

3 Removal and destruction by water treatment processes

4 Infection and disease

IV PROTOZOA

A Amoebae

1 *Endamoeba histolytica* - most important-with 4 waterborne outbreaks

a Only cysts important in water transmission

b Carrier rates relatively high in many places - highest in tropics and subtropics

c Outbreaks related to contamination of distribution system

d Important matters related to cysts:

1) Low cyst density in sewage, even lower in effluent, insignificant in effluent - receiving waters - explaining outbreaks related to contamination of distribution system; could be responsible for carriers.

2) Survival, removal, and destruction in water

B *Naegleria* - causing swimming associated meningo-encephalitis.

Acanthamoeba - causing nonswimming associated meningo-encephalitis.

C Other protozoa of some importance: *Trichomonas*, *Giardia*, *Balantidium coli*.

D Animal carriers?

V WORMS

A Sewage important in spreading ascariasis and other enteric round worm infections in Europe - contamination of vegetables by "artificial rain"

B Sewage pollution of pasture aggravating beef tapeworm infection in man

C Water in endemic areas of hookworm infections capable of spreading infective larvae which are highly resistant to free chlorine

D Cyclops intermediate host of fish tapeworm and guinea worm

E Swimmer's Itch - bird schistosome cercaria

VI EXAMINATION OF WATER FOR PATHOGENS

A Viruses Concentration - gauze-pad, continuous-flow, high-speed centrifugation, host-cell adsorption, MF filtration, magnetic iron oxide, polyelectrolyte and other media adsorption

B Bacteria - MF technique and selective media

C Protozoan Cysts - MF technique and examination of washings

D Worm Ova or Larvae - worm larvae demonstrable in cyclops; ova examination limited to sewage and sewage effluent

VII CONTROL

A Environmental vs. immunological - bringing human beings to environment or environment to human beings

B No methods of control 100% effective and yet practical; method with relatively high efficiency in removal or destruction of pathogen effective in control through impairment of the infection cycle - working toward elimination of the parasite

C Socio-economical factors to be taken into consideration for success

D In fact, water constituting a mode of transmission most susceptible to environmental control

E Control under emergency conditions

F Control of swimming associated amoebic meningo-encephalitis.

This outline was prepared by S. L. Chang, Etiology Criteria Dev. Br., WSRL, NERC, EPA.

EFFECTS OF POLLUTION ON FISH

I INTRODUCTION

A By what means do pollutants exert their effects?

B What is the relationship between water quality and water use by fishes?

C What is the reaction of fishes to domestic sewage?

D Is there any noticeable change in species composition of the population following pollution?

E Is there any genetic or environmental selection in favor of pollution resistant strains?

F Nearly any pollutant, given sufficient concentration and time, can kill as a direct toxicant. We are primarily concerned here with sub-lethal or chronic levels of pollutants. (Acute toxic levels and physiological mechanisms are treated elsewhere.)

II MECHANISMS OF DETRIMENTAL ACTION

A Inert silt may

1 Clog gills and smother eggs and fry,

2 Blind sight feeders and eliminate hiding places,

3 Smother food organisms,

4 Reduce oxygenation by smothering algae.

B Irritants may

1 Act as repellents,

2 Cause excessive mucous secretion and upset osmotic balance.

C Sub-lethal quantities of a host of environmental materials are constantly penetrating the bodies of fishes by various routes. We are generally not aware of their presence unless they:

1 Cause an observable effect on the fish,

2 Cause an effect on man by imparting off-taste or odor to fish flesh,

3 Are sought for and detected, e.g.; radioactive substances, DDT, mercury.

We can only speculate as to their undetected effects.

III ENVIRONMENTAL RELATIONSHIPS BETWEEN WATER QUALITY AND WATER USE BY FISHES

A Freshwater fishes sometimes spend their entire lives in a single body of water. Pollution of that body of water therefore impinges on them at every stage of their life cycle, and at every point in their various ecological relationships; such as, seeking food or escaping enemies.

B Migratory fishes on the other hand feed and grow up in one body of water (the ocean for anadromous species, fresh water for the catadronous eels), then travel a migration route (usually a river) to another body of water where they breed.

Pollution at either end of the route, or a pollution block along the migration route, may eliminate the species.

C What will affect one species adversely may be favorable for another.

1 Cold water species, such as various trouts, might be killed or eliminated by warmed water from a power plant which would in turn permit the survival of warm water species, such as certain basses, sunfishes, etc.

2 Benthic species (such as catfish, sculpins, or suckers, which live near the bottom) might be eliminated by a smothering

blanket of inert material which would not affect limnetic species inhabiting the open water areas (such as white and yellow bass, gizzard shad, or walleye). The limnetic species on the other hand might be inhibited by a dense turbidity which would hide their prey, suppress the growth of nutritious plankton, or clog their gills; this in turn being relatively harmless to the benthic group.

3 Likewise the shoreline hugging littoral forms (like pumpkinseed or bluegills) and profundal species (such as lake trout) might respond selectively to such factors as temperature or transparency.

IV RESPONSE OF FISHES TO SEWAGE AND SIMILAR WASTES

A These wastes in general are not toxic in themselves, but exert their effects on fishes directly.

B Oxygen Depletion

1 May lead to death at various stages in life history depending on circumstances.

2 May lower resistance to disease or increase sensitivity to intoxication.

3 May reduce ability to capture food or swim against current.

C May smother or kill normal food sources.

D May increase normal fish production through eutrophication.

E Usually changes normal population balance by driving out predatory types and encouraging scavengers.

F Reported to cause osteological and other pathological manifestations, such as the knothead condition of carps in the Illinois River.

V NATURAL SELECTION AND ACCLIMATIZATION TO POLLUTION

A Known biological mechanisms for selective breeding of pollution resistant strains operate in nature among fishes as among other organisms.

1 Studies of population genetics indicate that after some finite number of generations of population stress (e.g.; exposure to a given pollutant), permanent heritable resistance may be expected to develop.

2 If the environmental stress (or pollutant) is removed prior to the time that permanent resistance is developed in the population, reversion to the non-resistant condition may occur within a relatively few generations.

3 Habitats harboring populations under stress in this manner are often marked with the dead bodies of the unsuccessful individuals.

B Individual organisms on the other hand can over a period of time (less than one life cycle) develop a limited ability to tolerate different conditions, e.g.; pollutants:

1 With reference to all categories of pollutants both relatively facultative and obligate species are encountered (e.g.; euryhaline vs. stenohaline, eurythermal vs. stenothermal).

2 This temporary somatic acclimatization is not heritable.

C A given single-species collection or sample of living fishes may therefore represent one or more types of pollution resistance:

1 A sample of an original population which has been acclimated to a given stress _in toto._

2 A sample of the surviving portion of an original population, which has been "selected" by the ability to endure the stress. The dead fish in a partial fish kill are that portion of the original population unable to endure the stress.

3 A sample of a sub-population of the original species in question which has in toto over a period of several generations developed a heritable stress resistance.

D Any given multi-species field collection will normally contain species illustrative of one or more of the conditions outlined above.

VI POPULATION COMPOSITION RESPONSES TO POLLUTION

A Sewage pollution generally results in a reduction in the predatory types and their replacement by scavengers. Regions of severe oxygen depletion may be devoid of fish, or inhabited only by rough fish such as gar or carp. The general concept of a reduction of variety coupled with an increase in abundance in certain regions is as valid for fishes as for other groups.

B Population responses to toxic pollution are unpredictable except that reduction in variety is again almost sure to result.

REFERENCES:

1 California, State of. Water Quality Criteria, 2nd ed. Resources Agency of California, State Water Quality Control Board Publication No. 3-A. 1963.

2 Forbes, S.A., and Richardson, R.E. Some Recent Changes in Illinois River Biology. Bull. Ill. Nat. Hist. Sur., 13(6) 1919.

3 Jones, J.R. Erichsen. Fish and River Pollution. Butterworth's London, pp. 203. (1964)

4 Katz, Max, and Gaufin, A.R. The Effects of Sewage Pollution on the Fish Population of a Midwestern Stream. Midwestern Stream. Trans. Am. Fish. Soc. 82:156-165. 1952.

5 Moore, Emmeline. Stream Pollution and Its Effects on Fish Life. Sewage Works Journal. 4:159. 1932.

6 Naegele, John. A. Head, Dept. of Environmental Sciences, Univ. of Mass., Waltham Field Station, Waltham, Mass. Personal Communication. 1965.

7 Trautman, M.B. The General Effects of Pollution in Ohio Fish Life. Trans. Am. Fish. Soc. 63:69-72. 1933.

8 Mills, Harlow B.; Starrett, William C.; and Bellrose, Frank C. Man's Effect on the Fish and Wildlife of the Illinois River. Ill. Nat. Hist. Surv. Biol. Notes No. 57. 24pp. 1966.

9 Warren, Charles E. Biology and Water Pollution Control. W. B. Saunders Co. 434 pp. 1971.

This outline was prepared by H. W. Jackson Chief Biologist, National Training Center, EPA, Cincinnati, OH 45226.

INFLUENCE OF LAND RUNOFF ON WATER QUALITY

I Earthlife as we know it requires large quantities of water of the right quality, in the right place, at the right time (SINGER, 1968).

A Each person has high, average daily demands.

1 We each need to ingest about two quarts of water per day.

2 To sustain the food chain from the soil to the average person's stomach requires 2,500 gallons per day.

3 Adding the amount required to produce his clothing increases the average per capita water demands to 3,000 gallons per day.

4 The production of creature comforts requires other prodigious amounts.

a Refining 1 gallon of oil requires 18 gallons of water.

b Making a barrel of beer requires 300 gallons of water.

c Steam-powered generating plants use 191,700 gallons of water for each ton of coal that it burns.

d Since it takes 60,000 gallons of water to produce a ton of paper, a large paper mill needs as much water (60,000 × 1,000) as a city of 300,000 persons (300,000 × 200).

B The water supply of the Earth totals a staggering 326,000,000 cubic miles of water (NACE, 1967).

1 Oceans, inland seas, glaciers, and polar icecaps account for 99.37 percent of this supply.

2 Ground water, vadose water, and soil moisture make up 0.62 percent.

3 Fresh-water lakes and rivers have an average instantaneous amount equal to 0.01 percent.

4 The average, instantaneous, atmospheric moisture, the source of all precipitation upon the earth, amounts to only 0.001 percent of the earth's supply of moisture.

II Precipitation, extremely variable in amount, but usually of high quality, is the main source of the world's land runoff.

A In the United States, the average annual precipitation is relatively generous but it is unevenly distributed.

1 It averages about 30 inches per year.

2 It varies from virtually zero in the arid interior deserts to more than 150 inches at the tops of some of the high elevation mountain ranges of the Pacific Northwest.

B Cyclonic storms extend across large areas.

1 These "general" storms move from west to east across the United States.

2 These storms usually have fairly uniform characteristics but are subject to marked orographic effects.

C Convection-type storms tend to originate in certain areas and to traverse somewhat similar paths time after time.

1 Convection storms are relatively small, short, and intense and are much more common in some areas than in others.

2 Convection storms have peak intensities for short periods and these peaks decrease as longer duration periods and shorter frequencies (expected return periods) of the storms are used.

3 An estimated rainfall intensity for various duration periods and frequencies may be calculated from local records.

a To do this, the intensities of all storms on record must be listed in descending order, for the duration period in question, and an ascending order number assigned to each storm.

Example 1. Rainfall intensities at Cincinnati, Ohio during different peak periods of 1-year and 50-year frequency (return period) storms:

Duration Period (Minutes)	Intensities (inches per hour)	
	Once per year	Once in 50 years
5	3.94	9.40
10	3.18	8.60
15	2.55	7.42
30	1.65	5.20
60	1.02	2.60
120	0.61	1.51
1440	0.093	0.21

Example 2. Rainfall intensities at Cincinnati, Ohio during 5 and 120-minute periods of storms with different frequencies (return periods):

Frequency (Years)	Intensity (inches per hour)	
	5-min. period	120-min. period
1	3.94	0.61
2	4.51	0.72
5	5.50	0.90
10	6.38	1.14
25	7.38	1.34
50	9.40	1.51

Example 3. Five-minute rainfall rates, for a 52-year record, in descending order and their assigned, ascending order numbers:

Assigned Order Number (N)	Rainfall rate during the most intense 5-minute period of each storm (I--inches per hour)
1	9.72
2	7.32
3	7.32
4	6.60
5	6.48
6	6.31
7	6.23
etc.	etc.

b Choose F, the frequency (return period) desired and calculate N from the equation F = (M + 1)/N

where F = frequency (return period) in years,

M = years of record, and

N = order number.

Example 4. Determine N for a 10-year frequency and 5-minute intensity for the 52-year record used in Example 3.

10 = (52 + 1)/N; N = 53/10 = 5.3

Interpolation from the table shown in Example 3 using an N of 5.3 as calculated in Example 4 gives an intensity of 6.43 inches per hour.

D Contamination of Rainfall is usually relatively low.

Example 5. Constituent concentrations in a 0.35-inch rainstorm that fell July 12, 1964 at Cincinnati, Ohio:

Constituent	Concentration	Constituent	Concentration
pH	4.2	Nitrite Nitrogen (NO_2 - N)	0.0 mg/l
Alkalinity	*0.0 mg/l	Nitrate Nitrogen (NO_3 - N)	0.4 "
Total Solids	6.0 "	Ammonia Nitrogen (NH_3 - N)	0.4 "
Volatile Solids	3.6 "	Organic Nitrogen (Org - N)	0.8 "
Chemical Oxygen Demand (COD)	3.3 "	Total PO_4	0.26 "

* Milligrams per liter

E Rainfall frequency, duration, intensity, and amount are used in various combinations with other physical, chemical, and biological data to provide the basis for a number of engineering design purposes.

III The instantaneous volume of runoff draining from the world's land surface at any one time is a relatively small amount of the world's total water supply.

A Overland flow from an undisturbed rural environment is usually negligible.

1 Heavily vegetated areas cushion raindrop impact and keep the water clean.

2 Heavily vegetated areas have porous soils and are able to absorb clean water rapidly.

B Land use can greatly increase overland flow.

1 A good road surface is impervious to water.

2 Tillage practices cause greatly accelerated overland flow.

a Clean cultivation permits maximum raindrop impact and soil surface sealing.

b Cultivation causes soil compaction and it is also conducive to concrete frost formation.

c Up-and-down slope tillage concentrates overland flow and accelerates runoff.

d Logging, strip-mining, and other land use retards infiltration and augments runoff.

3 Numerous USDA soil and water conservation studies and practices provide information relative to the relation of land use and management to overland flow and subsurface drainage.

4 Preliminary PHS runoff/rainfall ratios for five small agricultural watersheds varied from 0.11 to 0.33 for a 2.21-inch rainstorm and from 0.20 to 0.56 for a 5.09-inch rainstorm.

C Drainage water from land areas differs greatly in quality compared to precipitation.

1 The agricultural watersheds at Coshocton, Ohio have provided some pertinent information and show that certain constituents have special significance.

Example 6. Quality of drainage water resulting from a rainstorm on one of the agricultural watersheds under study by the USDA and PHS at Coshocton, Ohio:

Constituent	Concentration
Turbidity	90 units
Color	260 "
pH	7.7
Alkalinity	100 mg/l
Hardness (as $CaCO_3$)	
Calcium	73. "
Magnesium	63. "
Total	136. "
Chloride	28. "
Chemical oxygen demand (COD)	159. "
Biochemical oxygen demand (BOD)	23. "
Total solids	392. "
Volatile solids	193. "
Suspended solids	56. "
Volatile suspended solids	31. "
Nitrite nitrogen (NO_2 - N)	0.1 "
Nitrate nitrogen (NO_3 - N)	4.9 "
Ammonia nitrogen (NH_3 - N)	0.4 "
Organic nitrogen	7.5 "
Total hydrolyzable PO_4	2.7 "
Ortho PO_4	
Total coliform bacteria	19,000. colonies per 100 ml
Fecal coliform bacteria	340,000 " " " "
Fecal streptococci bacteria	1,300 " " " "
Total organic chlorides	248 nanograms per liter
Lindane (possibly)	32 " " "
2, 4-D Ester (possibly)	141 " " "

a The nitrate, phosphate, and fecal coliform concentrations were high.

b The total organic chlorides could be of some concern.

2 Intensive sampling would be required in order to determine total loads of constituents lost in the runoff.

3 Livestock feedlots are troublesome in some areas (SMITH, 1968).

a The Kansas Board of Health ranks large livestock feedlots as that State's major pollution source.

b Some feedlots contain 10,000 animals.

c Ten thousand cattle have the pollution potential of 225,000 persons.

d Wastes from feedlots have caused fish kills and ruined water supplies of downstream communities.

D Urban development creates an artificial environment with widely varying water pollution potentials.

1 Use and treatment of urban lands and waters varies widely.

a There are 11,500 sewered communities in the 50 United States.

b These communities range in size from less than 0.5 to about 454 square miles.

c Their aggregate area equals about 43,100 square miles, or about 1.2 percent of the total area of the United States.

d Runoff coefficients in urban areas may range from 0.05 for lawns, gardens, and parks, to over 0.90 for roofs, streets, and other solidly-paved surfaces.

e "Design storm" tributary areas to storm sewers are made up of composites of these types of land use.

f About 37 percent of a residential, light-commercial type area is in roofs, streets, and parking lots and the remainder is in lawns, gardens, and parks.

g The entire surface area of some commercial urban areas may be covered with impervious materials.

2 About 83 percent of the 11,500 sewered U.S. communities (mostly the smaller communities) have "separate" sewers and the other 17 percent (mostly the large and old communities) have "combined" systems, or a mixture of "separate" and "combined" systems, with the mixture varying from almost all "separate" to almost all "combined".

a "Separate" sewer systems keep the sanitary sewage separated from the storm sewage so they can be handled independently.

b "Separate" systems have appealed to small communities because they could use a smaller conduit for the sanitary sewage if they used roadside ditches for storm water runoff.

c "Combined" systems have a common collector for both the sanitary and storm water sewage.

d During dry weather combined systems divert all the sewage and the intercepted drainage water to the treatment plant.

e During wet weather the volume of mixture delivered for treatment may be several times as large as the dry weather volume.

f During heavy storms an untreated volume of the mixture 100-200 times larger than that treated may overflow to the receiving streams.

g For some cities, "combined" systems are simpler and cheaper and they were often adequate years ago when all or at least part of the flow could be discharged without treatment.

3 Quantity of storm water runoff can be estimated by the "rational" method.

Q = CIA where:
C = runoff coefficient
I = rainfall intensity
A = drainage area

a The runoff coefficient (C) depends on surface conditions.

Example 7. Runoff coefficients for different kinds of urban surfaces.

Kind of Urban Surface	Runoff Coefficient
Mercantile	0.70 - 0.95
Commercial	0.60 - 0.85
Industrial	0.55 - 0.80
Residential (apartments)	0.5 - 0.7
Residential (single and double dwellings)	0.25 - 0.6
Parks	0.25
Undeveloped areas	0.05 - 0.25

b The rainfall intensity (I) is usually expressed as inches per hour and one inch of rainfall per hour is equivalent to a runoff flow of 1.008 cubic feet of water per second per acre.

c A "design" rainfall rate frequently used is that maximum which may be expected in the particular return period that has a duration period equal to the concentration time of the watershed point being considered.

Example 8. Using a 15-minute concentration time, a 0.5 runoff coefficient, and a rainfall rate (for a one-year return period and a 15-minute duration period) of 2.55 inches per hour (equivalent to 2.57 cfs) the flow per acre would be: (0.5) (2.57) (1) = 1.28 cubic feet per second. In comparison, a residential area sewage discharge at a population density of 10 per acre would be approximately (10) (100) (1/7.48) (1/86400) = 0.00155 cubic feet per second. In this case, the storm water runoff amounts to 826 times the sewage flow at this peak runoff rate.

4 Quality of drainage water from a residential, light-commercial area differs greatly in comparison to drainage from an agricultural area. (Compare Example 9 with Example 6).

Example 9. Constituent concentrations in urban land runoff at Cincinnati sampling point, July through December 1962 and March through September 1963 (WEIBEL, ANDERSON, and WOODWARD, 1964)

Constituent	Range	Mean	
Turbidity	30-1000. units	170.	
Color	10-380. "	81.	
pH	5.3-8.7	7.5	
Alkalinity	10-210 mg/l	59.	
Hardness (as $CaCO_3$)			
Calcium	24-200. "	63.	
Magnesium	2-46. "	15.	
Total	26-240. "	78.	
Chloride	3-35. "	12.	
Settleable solids	5-1200. "	210.	
Volatile settleable solids	1-290. "	53.	
Chemical oxygen demand (COD)	20-610. "	99.	
Biochemical oxygen demand (BOD)	2-84 "	19.	
Nitrogen (as N)			
Nitrite (NO_2)	0.02-0.2 "	0.05	
Nitrate (NO_3)	0.1-1.5 "	0.4	
Ammonia (NH_3)	0.1-1.9 "	0.6	
Organic	0.2-4.8 "	1.7	
PO_4 (Total soluble as PO_4)	0.07-4.3 "	0.8	
Bacterial counts	Number of colonies per 100 milliliters exceeded in designated percent of samples.		
	90%	50%	10%
Total Coliforms	2,900.	58,000.	460,000.
Fecal Coliforms	500.	10,900.	76,000.
Fecal Streptococci	4,900.	20,500.	110,000.

Example 10. Comparison of storm water runoff loads * and sanitary sewage loads *, pounds per acre per year: (WEIBEL, ANDERSON, and WOODWARD, 1964).

Constituent	1 Storm Water Runoff	2 Sanitary Sewage (raw)**	Ratio (Col. 1/Col. 2)
Settleable solids	730.	540.	1.35
Volatile settleable solids	160.	360.	0.44
Chemical oxygen demand (COD)	240.	960.	0.25
Biochemical oxygen demand (BOD)	33.	540.	0.06
Phosphate (PO_4)	2.5	27.	0.09
Total Nitrogen	8.9	81.	0.11

* Period July 1962 through September 1963, excluding January and February.
** From a residential-light commercial urban area.

a The importance of the BOD load carried by the storm water runoff shown in Example 10 is probably much greater than that shown by the 0.06 ratio of storm water load to raw sanitary sewage load.

b Sewage treatment would likely remove 90 percent of the raw sanitary sewage BOD.

c The 33 pounds per acre per year load of BOD in the storm water runoff would then be equivalent to 0.6 of the 54 pounds per acre per year of BOD load in the treated sewage effluent.

d The storm water runoff shown in Example 10 is from a relatively "clean" type of land use. Densely populated and industrial urban areas would probably produce more heavily polluted storm water runoffs.

e Studies of stream water quality indicate that coliform bacteria counts increase as turbidity increases. (GELDREICH, 1966) (KUNKLE and MEIMAN, 1968).

f Turbidity varies almost directly with streamflow. Therefore, much of the bacterial load probably arises from surface wash.

g Drainage from a non-urban area such as the Lake Mendota watershed can furnish sufficient nutrients to fertilize the water to the nuisance aquatic growth-producing level. (MACKENTHUN, 1965 p. 11)

h Some rural water supplies may contain 500 milligrams of nitrate nitrogen per liter. (MACKENTHUN, 1965 p. 11)

i On an agricultural watershed near Coshocton, Ohio, two storms with 2.21 to 5.09 inches of rainfall per storm produced 6,600 to 76,300 gallons of runoff per acre. (MACKENTHUN, 1965 p. 12)

j Phosphate (PO_4) in this runoff water ranged from 0.05 to 0.42 pounds per acre.

k Total nitrogen (N) ranged from 0.20 to 6.12 pounds per acre.

l The use of pesticides has increased agricultural production and improved mankind's health and comfort. (BOGAN, OKEY, and VARGAS, 1961) (HOFFMAN, 1959)

m Pesticides will have to be used with increasing care in order to avoid tragic, secondary effects, however. (WEBB, 1960) (WORRELL, 1960) (GILETT, 1970)

n Pesticides already occupy a prominent position among the list of fish-killing water pollutants. (MCKEE and WOLF, 1963) (PRESSMAN, 1963) (PUBLIC HEALTH SERVICE, 1961) (COTAM, 1960) (BARRY, 1961)

o The compounds DDD, DDE, DDT, chlordane, and endrin were found regularly in samples of both water and biota taken during the pesticide monitoring of the aquatic biota of the Tule Lake National Wildlife Refuge and the occurrence of endrin was directly associated with contaminated irrigation return water supplying the Refuge Lakes. (GODSIL and JOHNSON, 1968)

p Many studies show that some herbicide will appear in nearly all streams which flow by or through treated areas. (NORRIS, 1967)

q A water treatment of soil-applied picloram pellets for the control of chaparral brush resulted in the movement of detectable amounts of picloram into the stream water. (DAVIS, INGEBO, and PASE, 1968)

r The herbicide amitrole, aerially sprayed on 100 acres of a headwater sub-basin of a steep, clearcut, forest watershed in a silvicultural attempt to control salmonberry, was found in measurable amounts in samples of water near the downstream edge of the sprayed area during and for 5 days after spraying. (MARSTON, SCHULTS, SHIROYAMA, and SNYDER, 1967)

5 "Combined" sewer problems may be even more troublesome than those of separate systems.

a Treatment plants are seldom large enough to handle all the volume produced during heavy storms.

b The quantities and flow patterns are comparable to separated storm water flows. However, usually during storm periods, combined systems can handle no more than 1.5 to 5 times the normal dry weather flow.

c When the stormflows exceed this amount (see the 826-fold increase shown in Example 8) some, most, or all of the flow must overflow to the effluent receiving streams.

d During the 1964 Christmas floods many of the sewage treatment plants in the Pacific Northwest were completely inoperative. (MARSTON and NIELSON, 1967)

e Elsewhere it has been claimed that as much as 95 percent of the sanitary sewage overflows during some storms.

f "...about 1/3 of the city's annual production of sewage solids overflowed without treatment although only 2 to 3 percent of the sewage volume actually overflowed..."

g Some "combined" systems have been custom-tailored to provide local relief at certain critical points by diverting part of the load to other points of overflow.

h Most storm sewers and certain parts of most combined systems are of an extremely localized nature.

i The number of overflow points varied from 1 to 42 among 30 cities.

j Little specific qualitative information has been determined for combined sewer overflows.

Example 11. Quality of a combined sewer overflow:

pH	7.1	
Chloride	619.	mg/l
Total solids	400.	"
Volatile solids	144.	"
Settleable solids	203.	"
Sand	76.	"
Dissolved oxygen	6.9	"
Oil and grease	33.	"
Biochemical oxygen demand	59.	"

IV Nearly all of the 17 percent of the United States supply of water which is withdrawn, used and returned to the waterways is polluted to some degree. (SINGER, 1968)

A This restricts its further use and contaminates the receiving waters.

B The streams' self-purification processes are unable to cope with today's massive waste burdens and complex effluents.

C Effective waste management is needed to protect clean waters and to upgrade polluted waters so that they may be reused. The land surface can be used as one method of waste treatment and disposal.

1 The overlying soil and the underlying geological strata may be thought of as a three-dimensional, anisotropic, chromatographic column capable of adsorbing and removing solutes from a percolating solution. (BAILEY, 1968)

a If the interactions between different nitrogen and phosphorus forms in the effluent and soils and geological strata are known, it is possible to predict the chromatographic behavior of the "soil waste treatment system."

b Using such information it would be possible to set forth the criteria for selecting sites of "soil waste treatment systems."

c Similarly it would be possible to indicate how those soils, lacking the necessary critical properties, can be modified to serve as "soil waste treatment systems."

2 An intensive study at Pennsylvania State University has given much factual information on the efficiency of the soil as a filtering medium (PENNYPACKER, SOPPER, and KARDOS, 1967)

a Overland flow causes soil erosion and washes silt and toxicants into the streams.

b Avoid overland flow by increasing infiltration, interflow and sub-flow. (MARSTON and WHIPKEY, 1964). Use sprinkler applications; disperse and control overland flows by the use of grassed waterways.

REFERENCES

1 Bailey, George W. Role of Soils and Sediment in Water Pollution Control, Part I. Dept. of the Interior, FWPCA, Southeast Water Laboratory. (1968)

2 Barry, E.M. Fish Kills by Chemicals. Interstate Commission on the Potomac River Basin. (1961)

3 Bogan, R.H., Okey, R.W. and Vargas, D.J. Pesticides in Natural Waters. Research 14:269 (1961)

4 Cotam, C. Pesticides and Water Pollution Proc. National Conference on Water Pollution, PHS. (1960)

5 Davis, E.A., Ingebo, P.A., and Pase, C.P. Effect of a Watershed Treatment with Picloram on Water Quality. USDA, FS, Rocky Mountain Forest and Range Experiment Sta. Research. Note RM-100. (1968)

6 Geldreich, E.E. Sanitary Significance of Fecal Coliforms in the Environment. DI, FWPCA, Research Series #WP-20-3. (1966)

7 Godsil, Patrick J. and Johnson, William C. Residues in Fish, Wildlife, and Estuaries. Pesticides Monitoring Journal 1(4):21-26. March 1968.

8 Hoffman, C.H. Are the Insecticides Required for Insect Control Hazardous to Aquatic Life? RATSEC, PHS, Biol. Problems in Water Pollution. Tech. Report W 60-3, 51. (1959)

9 Kunkle, Samuel H. and Meiman, James R. Sampling Bacteria in a Mountain Stream, Colorado State University. Hydrology Paper #28. (1968)

10 Mackenthun, Kenneth M. Nitrogen and Phosphorus in Water--an Annotated Selected Bibliography of Their Biological Effects. HEW, PHS, DIV. of Water Supply and Pollution Control. (1965)

11 Marston, Richard B. and Nielson, Lyman J. Watershed and River Basin Management Related to Pollution Problems During High Water. DI. FWPCA. PNWL. (1967)

12 Marston, Richard B., Schults, Donald W., Shiroyama, Tamotsu and Snyder, Larry V., Amitrole Concentrations in Creek Waters Downstream from an Aerially Sprayed Astoria Watershed Sub-basin. DI. FWPCA. PNWL. (Preliminary Report) (1967)

13 Marston, Richard B. and Whipkey, R.Z. Natural Waterworks, Ohio Woodlands. 2(3):4-5. (1964)

14 McKee, Jack Edward and Wolf, Harold W. Water Quality Criteria. California State Water Quality Control Board. Pub. 3-A. (1963)

15 Nace, Raymond L. Are We Running Out of Water. DI. Geological Survey. Circ. #536. (1967)

16 Norris, Logan A. Chemical Brush Control and Herbicide Residues in the Forest Environment. Symposium Proceedings. Oregon State University. (1967)

17 Pennypacker, S.P., Sopper, W.E. and Kardos, L.T. Renovation of Wastewater Effluent by Irrigation of Forest Land. Journal Water Pollution Control Fed. 39(2):285-296. (1967)

18 Pressman, Ralph. Pesticides. California State Water Quality Control Board, Sacramento, Calif. Pub. #3-A. (1963)

19 Public Health Service. Introductory Report, The Columbia River Basin Project for Water Supply and Water Quality Management. Region IX, Portland, Oregon. (1961)

20 Singer, Fred S. Water--The Dept. of the Interior's Systematic Management of a Resource. Remarks, Am. Astronautical Society, Las Vegas, Nevada. News Release, April 10, 1968.

21 Smith, T.R. Effect of Agriculture on Water Quality. Remarks, Rotary Club, Mt. Carmel, Illinois, February 5, 1968.

22 Webb, B. The Use of Herbicides in Relation to Fish and Wildlife. Idaho Wildlife Rev. 12:5, 11. (1960)

23 Weibel, S.R., Anderson, R.J. and Woodward, R.L. Urban Land Runoff as a Factor in Stream Pollution. Journal Water Pollution Control Federation. 36(7)914-924, July 1964.

24 Worrell, A.C. Pests, Pesticides, and People. American Forest Magazine, 66(7):39-61, July 1960.

25 Gilett, James W., Editor. The Biological Impact of Pesticides in the Environment. Environmental Health Sciences Center, Oregon University. 1970.

ACKNOWLEDGEMENT

Certain portions of this outline contains training material from prior outline by Samuel R. Weibel.

This outline was prepared by Dr. Richard B. Marston, Water Quality Management Specialist, Northwest Region, Federal Water Pollution Control Administration, Portland, Oregon.

GROUND WATER QUALITY STUDIES

I INTRODUCTION

Ground water forms an important part of the Nation's water supply. In 1960 water withdrawn from the ground accounted for about one-sixth of the total water withdrawn for use.(1) The true importance of ground water as a resource is probably not reflected by this figure because the ground water supply is capable of very much greater development in many areas.(2)

The survey of the quality of ground water in its natural state and the deterioration of ground water quality are the problems with which this outline is concerned. In order to evaluate a ground water quality problem it is necessary to consider the basic principles of ground water hydrology.

II GROUND WATER HYDROLOGY(3, 4, 16)

A Definition of Ground Water

Ground water is the water in the saturated zone of the soils, alluvium, and rocks that form the crust of the earth.

B Occurrence of Ground Water

1 The hydrologic cycle

Ground water is not an independent water resource, but is one phase of the hydrologic cycle, which is a continuous cycle of evaporation and transpiration, precipitation, runoff, infiltration, and ground water flow (Figure 1).

2 Ground water aquifers

A ground water aquifer is water bearing geologic horizon (bed, stratum, deposit, formation, etc.) which is sufficiently permeable to permit reasonably free movement of water. In contrast, an aquiclude may contain water, but it is relatively impermeable. The majority of developed ground water aquifers are composed of unconsolidated sand and gravel. Limestones with solution or fracture permeability, permeable zones in volcanic rocks, and consolidated sandstones and conglomerates are also important ground water aquifers. Other rock types (clays, shales, siltstones, metamorphic rocks, etc.) may yield small quantities of water, but are generally poor aquifers.

A water table o. unconfined aquifer is open to the surface throughout its areal extent, whereas a confined aquifer lies between impermeable beds. Confined aquifers under pressure are artesian aquifers.

3 Some aquifer properties

a Porosity is the ratio of void space to total volume.

b Permeability (transmissibility) is a measure of the ease of flow through porous media.

c Storage coefficient is the volume of water an aquifer takes into storage per unit surface area per unit change in pressure normal to that surface.

d Specific yield is the ratio of the volume of water that can be drained from a volume of rock by gravity to the total volume of the rock. It is also known as the effective porosity of the rock.

4 Ground water basins and their relation to surface waters

A ground water basin may be defined as a physiographic unit containing a ground water aquifer or aquifers capable of yielding a substantial water supply.

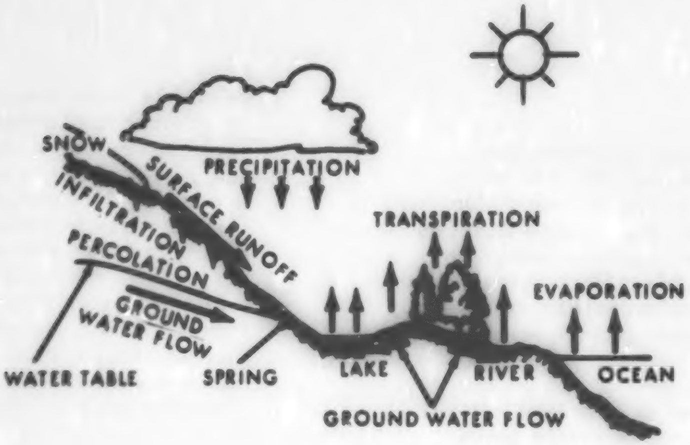

SNOW
PRECIPITATION
SURFACE RUNOFF
INFILTRATION
PERCOLATION
TRANSPIRATION
EVAPORATION
GROUND WATER FLOW
WATER TABLE
SPRING
LAKE
RIVER
OCEAN
GROUND WATER FLOW

Figure 1. THE HYDROLOGIC CYCLE$^{(3)}$

Many ground water basins are intimately related to the drainage basins of surface water streams and optimum utilization of water resources requires conjunctive management of surface and ground water supplies.$^{(17, 18)}$

5 Distribution of ground water in the United States

Thomas$^{(5)}$ has divided the conterminous United States into 10 ground-water regions. Each region has physiographic, geologic, and climatic features which cause its hydrologic features to contrast with those of adjacent regions.

In a broader sense, the distribution of water in the United States can be categorized into the area where precipitation exceeds potential evapotranspiration (roughly the 31 Eastern States) and there is a water surplus and the area where the reverse situation exists (roughly the 17 Western States) and there is a water scarcity. (Figure 2)

C Ground Water Movement$^{(3, 6, 7, 8, 16)}$

1 Fundamental principles governing fluid flow through ground water aquifers.

a Darcy's empirical law in its most elementary form is (Figure 3).

$$Q = KA \frac{\Delta h}{\Delta L} = Av \qquad (1)$$

Q = unit rate of flow
A = cross sectional area normal to flow path
v = apparent fluid velocity
Δh = fluid potential loss along flow path
ΔL = length of flow path
K = coefficient of permeability or hydraulic conductivity

b Coefficients of permeability (hydraulic conductivity) and transmissibility. From equation (1)

$$K = \frac{Av}{A} \quad \frac{\Delta L}{\Delta h} = v \quad \frac{\Delta L}{\Delta h} \qquad (2)$$

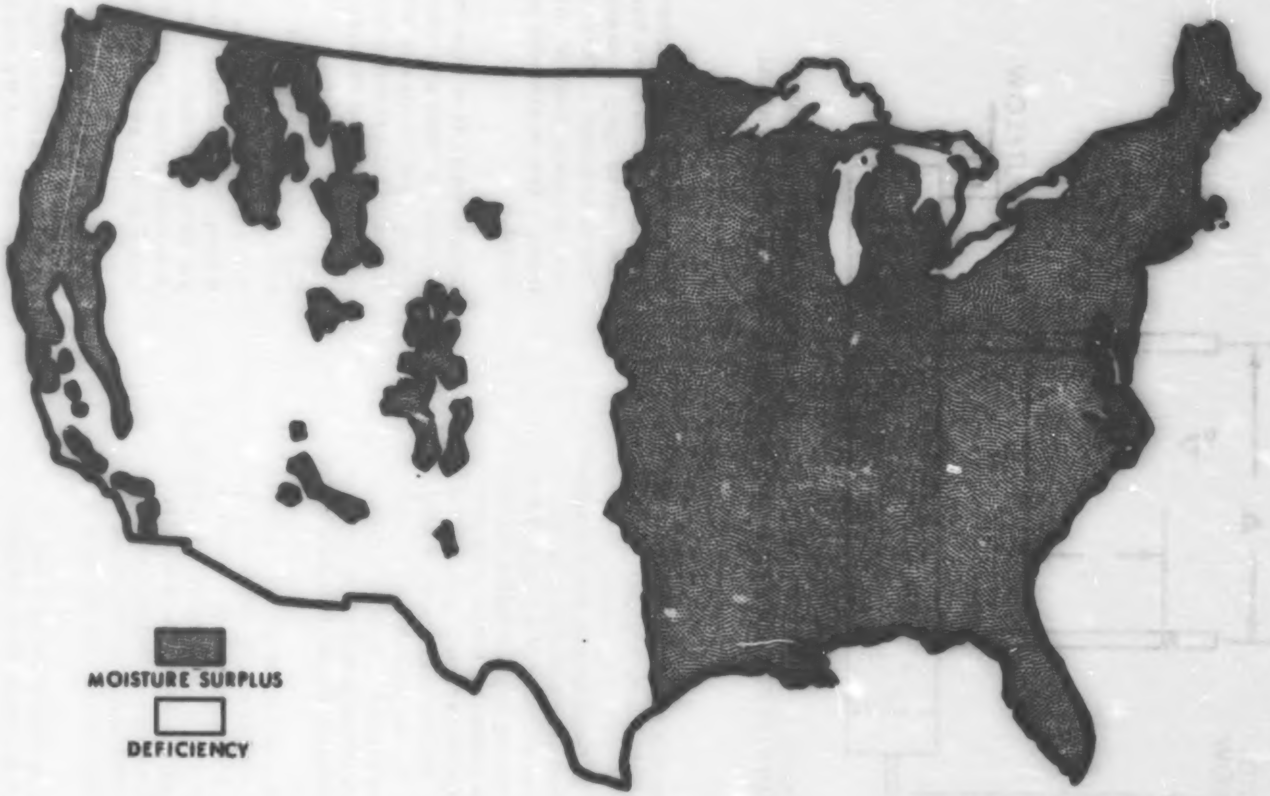

MOISTURE SURPLUS
DEFICIENCY

Figure 2. MAP OF THE UNITED STATES SHOWING AREAS OF MOISTURE SURPLUS AND DEFICIENCY AS OUTLINED BY THORNTHWAITE(14)

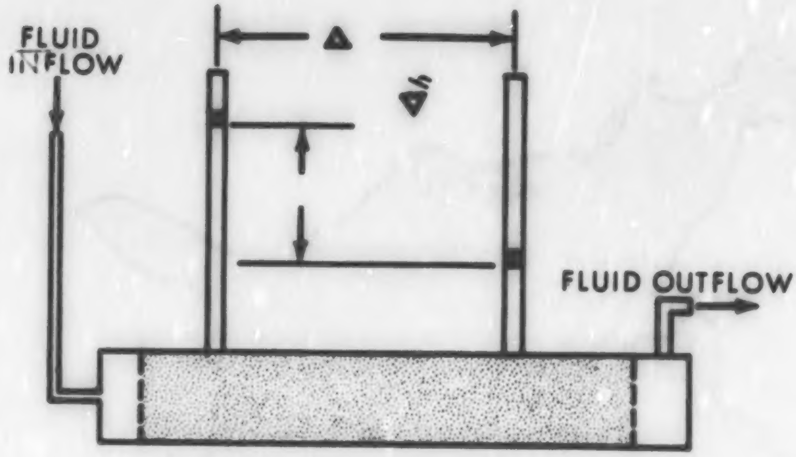


Figure 3. FLUID FLOW THROUGH A SAND PACKED COLUMN PIEZOMETERS INDICATE HEAD LOSS Δh ALONG AN INTERVAL ΔL

based on this equation, the units of K are length/time, e.g., ft/day.

The U. S. Geological Survey standard coefficient of permeability K_s is defined as the flow of water at 60°F in gallons per day through a medium having a cross-sectional area of 1 ft^2 under a hydraulic gradient of 1 ft/ft. For natural aquifers the value of K_s varies between 10 and 5000 gal/day/ft^2. A typical clean sand and gravel aquifer, might have a permeability K_s of 1000 gal/day/ft^2.

The permeability of ground water aquifers is commonly expressed in terms of transmissibility which is permeability multiplied by the aquifer thickness. A 100-ft thick aquifer with a field permeability of 1000 gal/day/ft^2 would have a transmissibility of 100,000 gal/day/ft.

Efforts to derive Darcy's law on a fundamental basis have not been entirely successful, but it has nevertheless been found to be valid for a wide range of flow conditions.

Ground water flow is generally laminar. Darcy's law has been found not to apply in cases where flow is turbulent. There is apparently no lower limit of validity for Darcy's law in granular porous media.

c Ground water flow must obey the equation of continuity which for steady flow (no change with time of any physical variable) in combination with Darcy's law reduces to

$$\frac{\partial^2 h}{\partial x^2} + \frac{\partial^2 h}{\partial y^2} + \frac{\partial^2 h}{\partial z^2} = 0 \qquad (3)$$

The solution of equation (3) for the appropriate boundary conditions yields a mathematical model which describes ground water flow behavior, under steady state conditions.

d Unsteady flow conditions in which there is a change in physical variables with time obeys a more complex form of the continuity equation. The unsteady flow of water in a compressible confined aquifer of uniform thickness b with a storage coefficient s is governed by the approximate partial differential equation.

$$\frac{\partial^2 h}{\partial x^2} + \frac{\partial^2 h}{\partial y^2} + \frac{\partial^2 h}{\partial z^2} = \frac{s}{Kb} \; \frac{\partial h}{\partial t} \quad (4)$$

where t is the time since pumping began.

e Unsaturated flow,$^{(19)}$ which is an important concept in ground water recharge through pits or spreading basins, irrigation practice, rain water infiltration, etc., can, in some cases, be described in terms of Darcy's law. In multiphase flow, however, the coefficient of permeability must be known for both air and water and the transport of water through the system related to the degree of water saturation. The rate of water flow through a system 50 percent water saturated is much less than half the flow rate in a saturated system under the same pressure gradient.

f No satisfactory theory exists for the movement of fluids through fractured rocks or cavernous limestone. On a practical basis, if the voids in such rocks are uniformly distributed on a large scale, flow equations based on Darcy's law may be found to apply.

2 Ground water flow rates and patterns can be predicted for homogeneous saturated porous media by the use of mathematical equations and physical models.

The extension of mathematical equations to nonideal aquifers is difficult, but physical models can sometimes be constructed if the characteristics of the aquifer are well enough known. Types of physical models are sand models, electrical models, viscous fluid models, and membrane models.

The rate of movement of ground water depends on the permeability of the aquifer in question and the hydraulic potential gradient which exists in the aquifer (Equation 2). The natural rate of movement of ground water in highly permeable sands of the Upper Glacial Aquifer of Long Island, N. Y., has been found to be more than a foot per day. Flow rates higher than 100 feet per day have been reported from field tests in other areas. On the other hand, the rate of movement in most aquifers would be a few inches per day and in deeply buried relatively impermeable aquifers may be so small as to be difficult to measure.

High ground water flow rates can occur in the vicinity of discharging or recharging wells where an appreciable increase in hydraulic gradient occurs.

Patterns of movement of ground water can be readily visualized through the use of a contour map of the piezometric surface along with the flow lines which will be perpendicular to the equipotential lines (Figure 4). Such maps can be constructed from observation well data to show existing field conditions. Such maps can also be constructed on the basis of mathematical equations or model studies to show probable future flow patterns.

3 Movement and distribution of contaminants

Contaminants in ground water travel in the same manner as the ground water in which they are dissolved or suspended, but some factors not normally considered in ground-water movement enter into the movement and distribution of contaminants.

Hydrodynamic dispersion is the mechanical mixing that takes place at the boundary between two miscible fluids as they move through porous media. Dispersion and molecular diffusion cause lateral (Figure 5) and longitudinal spreading of a contaminant that has been

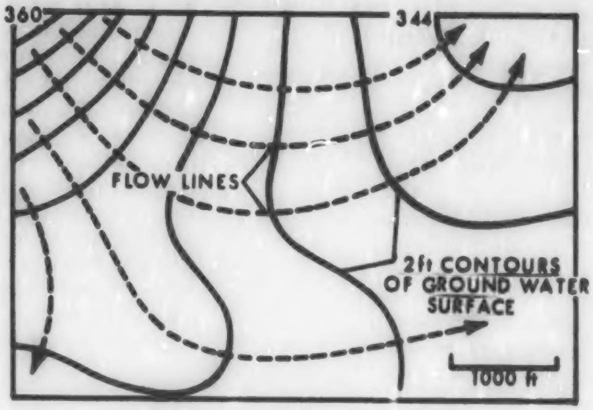

360
344
FLOW LINES
2ft CONTOURS OF GROUND WATER SURFACE
1000 ft

Figure 4a. CONTOUR MAP OF A GROUND WATER SURFACE AND SHOWING FLOW LINES(3)

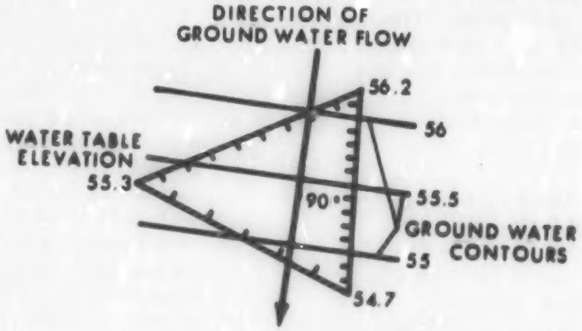

DIRECTION OF GROUND WATER FLOW
56.2
56
WATER TABLE ELEVATION
55.3
90°
55.5
GROUND WATER CONTOURS
55
54.7

Figure 4b. ESTIMATE OF GROUND WATER CONTOURS AND FLOW DIRECTION FROM WATER TABLE ELEVATIONS IN THREE WELLS(3)

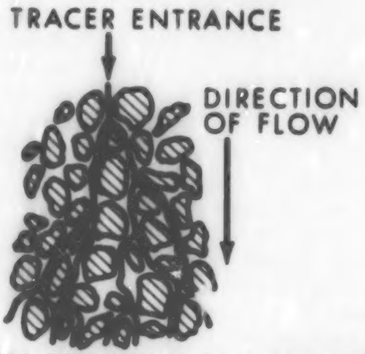

TRACER ENTRANCE
DIRECTION OF FLOW

Figure 5. LATERAL DISPERSION OF A TRACER ORIGINATING FROM A POINT SOURCE IN A POROUS MEDIUM(3)

introduced into an aquifer. That is, because of dispersion and diffusion the leading edge of a slug of contaminant will travel at a rate higher than the average flow velocity, and the outside edges will expand constantly outward from the center. Dispersion can be observed to occur on a microscopic scale in sand-packed models and on a macroscopic scale in natural aquifers. Coefficients of dispersion observed in field studies are from several times to several magnitudes larger than dispersion coefficients observed in small-scale experiments. Dispersion in natural aquifers is probably largely caused by heterogeneous permeability distribution. Molecular diffusion contributes importantly to the total mixing process only at low-flow velocities (probably less than one foot per day.)

Contaminants in ground water can be removed during flow through the aquifer by filtration, ion exchange, sorption, chemical reaction with aquifer minerals, chemical reaction with aquifer water, natural decay (radioactive contamination), and biological degradation.

D Ground Water Quality(22)

1 Measures of ground water quality are chemical, physical, and biological characteristics. The ground water properties measured in physical analysis include temperature, color, turbidity, odor, and taste. Biological analysis usually consists of tests for the presence of coliform organisms, but in special circumstances it may be of interest to analyze for other bacteria and perhaps for virus.

A complete chemical analysis of a ground water sample would include the determination of all inorganic constituents and in some pollution surveys the analysis of organic constituents as well. In reality, the usual procedure would be to analyze for total dissolved solids, pH, and conductance, and to analyze for the specific inorganic ions which are important in the individual case.

Organic properties which might be analyzed for are COD, BOD, CCE, ABS, chlorinated hydrocarbons, etc.

2 Standards of acceptability are determined by the intended use. Quality criteria have been developed for drinking water,(9) agriculture,(10, 20, 21) and various industrial uses. Special criteria could be developed for cases where existing standards are not satisfactory.

3 Ground water quality deterioration can occur in many ways as indicated in Table 1. Extensive contamination of ground water aquifers by irrigation return water and sea water intrusion(11, 23) has occurred in many cases.

Gross contamination from domestic or industrial wastes is less likely to occur, but the Upper Glacial Aquifer on Long Island, N. Y.,(12) and shallow aquifers in the suburbs of Minneapolis and St. Paul(13) have been contaminated over wide areas by individual waste disposal systems.

III A GROUND-WATER QUALITY SURVEY

A Defining Problem

1 General considerations

There are essentially two conditions which can exist when attempting to define the quality of ground water which has been polluted.

a The source of pollution is established and the degree and extent of pollution is to be determined.

b The degree and extent of pollution must be determined along with locating the pollutional source.

Table 1. CAUSES OF DETERIORATION OF GROUND WATER QUALITY(15)

Contamination and Pollution

- Domestic and Municipal Sewage
- Industrial Wastes
  - Organic wastes
    - Food processing
    - Lumber processing
  - Mineral wastes
    - Metal processing industries
    - Mining and ore extraction industries
    - Oil industries
    - Chemical industries
    - Miscellaneous
  - Cooling water
- Solid and Semisolid Refuse

Degradation

- Effects of Development, Use, and Reuse of Water
  - Irrigation return water
    - Surface drainage
    - Percolation
  - Interchange between aquifers due to improperly constructed, defective, or abandoned wells
  - Interchange between aquifers due to differentials in pressure levels resulting from excessive withdrawal
  - Overdraft conditions
    - Sea water intrusion
    - Salt balance
    - Upward or lateral diffusion of connate brines and/or juvenile water due to overpumping
  - Contamination from the surface due to improperly constructed wells
- Natural Causes
  - Inflow and/or percolation of juvenile water from highly mineralized springs and streams
- Other Causes
  - Accelerated erosion
  - Mineralization resulting from plant transpiration and/or evaporation

2 Preliminary identification of potential pollution sources

In any ground-water pollution study it is well to be aware of the various means by which pollution can occur and evaluate these on an areal basis to guide the study in its conception.

a Infiltration - pollutants carried to the land can find their way to fresh ground water. Practices leading to pollution by this means include:

1) Holding ponds such as brine evaporation oils, sewage lagoons, waste detention ponds, etc.

2) Areal dispersion such as animal feed lots, spray or flood irrigation with wastes, dump grounds, land fill operations, sludge drying, etc.

b Wells - pollution may enter a fresh water horizon through various types of wells:

1) Inadequately plugged abandoned wells where pollutants can pass from a lower or higher polluted zone to a fresh water strata

2) Leaking disposal wells

3) Improperly constructed wells permit contamination from the surface

c Natural - in its travel to and through aquifers water necessarily contacts minerals which go into solution. Therefore, natural pollution usually results from inorganic ions such as chloride, nitrate, sulfate, etc.

d Intrusion - a decline in ground-water tables whether natural or resulting from the activities of man can result in the movement of degraded waters into the zone of fresh water. An example of salt water intrusion is along the Gulf coast where extensive pumping is causing Gulf waters to move inland.

B Planning Survey

In planning a ground water survey the following steps might be a logical outline of procedures:

1 Determine the goals of the study; for example locating the source and degree of pollution.

2 Investigate sources of available data relative to geology and possible sources and types of pollution. This information includes the locations of existing water wells, geology, and the presence and locations of, for example, salt water disposal pits, feed lots, septic tanks, etc. Pertinent information may be obtained from a number of sources:

a Geological survey

b State and County officials, i.e. engineers, agricultural agents, etc.

c Local water well drillers

3 If available, sample appropriate water wells and possible pollution sources to gain background or base line information on quality parameters. Determine the presence of any trace element that might be used as a "finger point" of the problem, i.e. boron when dealing with an oil field brine.

4 Develop the project plan

Upon completion of the planning surveys during which all available data was compiled, the project plan should be developed which reflects:

a Available funds

b Available personnel and commensurate capabilities

c Time allocation

The plan may include the construction of observation wells for sampling or water level recording. It may include the sampling of existing water wells. In either case provisions must be made for land use for this purpose.

The project plan should reflect:

a Project need

b Project goals

c Project cost in terms of equipment, personnel, and supplies

d Exact location and schedule of sampling

e The expected approach for converting collected data into a form which will satisfy project goals.

REFERENCES

1 MacKichan, K. A., and Kammerer, J. C. Estimated Use of Water in the United States. 1960. U. S. Geol. Survey Circular 456. 26 pp. 1961.

2. McGuiness, C. L. The Role of Ground Water in the National Water Situation. U. S. Geol. Survey Water Supply Paper 1800. 1121 pp. 1963.

3 Todd, D. K. Ground Water Hydrology. Wiley and Sons, Inc. New York. 335 pp. 1959.

4 Meinzer, O. E. (ed.). Hydrology. McGraw-Hill Book Co., Inc., New York. 712 pp. 1942.

5 Thomas, H. E. Ground Water Regions of the United States - Their Storage Facilities. U. S. 83rd Cong., House Interior and Insular Affairs Comm., The Physical and Economic Foundation of Natural Resources. Vol. 3. 78 pp. 1952.

6 Collins, R. E. Flow of Fluids Through Porous Materials. Reinhold Publishing Corp., New York. 270 pp. 1961.

7 Muskat, M. Flow of Homogeneous Fluids Through Porous Media. J. W. Edwards, Inc., Ann Arbor, Michigan. 763 pp. 1937.

8 Scheideggar, A. E. The Physics of Flow Through Porous Media. 2nd Ed. The Macmillan Co., New York. 313 pp. 1960.

9 U. S. Dept. Health, Education, and Welfare, Public Health Service, Public Health Service Drinking Water Standards, 1962. U. S. Govt. Printing Office, PHS Pub. No. 956. 61 pp. 1962.

10 Wilcox, L. V. Classification and Use of Irrigation Waters. U. S. Dept. Agr. Circular 969. Nov. 1955.

11 State of California, Dept. of Water Resources, Sea Water Intrusion in California, Bull. No. 63. Nov., 1958.

12 Flynn, J. M., Jr. Impact of Suburban Growth on Ground Water Quality in Suffolk County, New York, In Ground Water Contamination, U. S. PHS Tech. Rept. W61-5. 1961.

13 Woodward, R. L., Ground Water Contamination in the Minneapolis and St. Paul Suburbs In Ground Water Contamination. U. S. PHS Tech Rept. W61-5. 1961.

14 Thornthwaite, C. W. An Approach Toward a Rational Classification of Climate. Geog. Review. V. 38. pp. 56-75. 1948.

15 Calif. State Water Resources Board, Water Utilization and Requirements of California, Bull. 2. Vol. 2. Sacramento. 358 pp. 1955.

16 Dewiest, R. J. M., Geohydrology: New York, John Wiley & Sons, Inc. 366 p. 1965.

17 Morgan, R., The Role of Ground Water in Comprehensive River Basin Planning: Ground Water, V. 3, No. 2, April, p. 16-18. 1965.

18 Bittinger, M. W., The Problem of Integrating Ground-Water and Surface-Water Use: Ground Water, V. 2, No. 3, July, p. 33-44. 1964.

19 Bouwer, H., Unsaturated Flow in Ground Water Hydraulics: Am. Soc. Civil Engineers Proc. Paper 4057, V. 90, No. HY5, p. 121-144, Sept. 1964.

20 Richards, L. A., (ed.), Diagnosis and Improvement of Saline and Alkali Soils: Agricultural Handbook 60, U.S. Dept. Agric., Washington, D.C., 160 p. 1954.

21 Lunt, O.R. (ed.) Proceedings Agricultural Water Quality Research Conference: California Water Resources Center Report No. 5, 69 p. 1963.

22 McKee, J.E., and Wolf, H.W., Water Quality Criteria (2nd ed.): The Resources Agency of California, State Water Quality Control Board Pub. No. 3-A., 548 p. 1963.

23 Wait, R.L., and Callahan, J.T., Relations of Fresh and Salty Ground Water Along the Southeastern U.S. Atlantic Coast: Ground Water, V. 3, No. 4, p. 3-17. 1965.

This outline was prepared by Don L. Warner, Former Sanitary Engineer, Technical Services, Ohio Basin Region, FWQA. Reviewed and revised by the Ground Water Research Program Staff, Robert S. Kerr Water Research Center, Ada, Oklahoma.

THE EFFECTS OF ORGANISMS ON POLLUTION AND THE ENVIRONMENT

I INTRODUCTION

A Pollution is often studied as a factor affecting the biota, but it is equally important to recognize the environmental changes produced by the biota.

B According to Westlake, under many conditions "....the environment is almost as much a product of the community as the community is of the environment."

II SOME ENVIRONMENTAL EXAMPLES

A Diatom Blooms

"...Asterionella (an oil storage alga) produces an autotoxin (autoantibiosis) which will inhibit itself, may stimulate or inhibit other species that store oil, but always stimulate algae that store starch. For example, Asterionella may produce a bloom and inhibit itself, but stimulate a population of Synedra. These oil storage algae produce a substance that stimulates a starch-storing alga, Coelastrum, which may stimulate another starch-storing organism, Cosmarium, and they, in turn, stimulate an oil storing species of Dinobryon." Patrick.

B The altered structure of the plankton community due to the introduction of the alewife

C Fecal deposit feeders in the estuarine environment

D Particle feeders are successful in the pipe clogging community and are generally (Sebestyén):

1 Sessile

2 Suspension feeders

3 Have motile larvae

4 Resistant stages

E Biogeochemical Cycles

In terms of biomass and energy flow, the mussel Modiolus (Figure 1) is a relatively minor component of the marsh community. However, they have been demonstrated to have a major effect on the recycling and retention of valuable phosphorus; thereby maintaining fertility and production of autotrophs (Odum).

F Sudd (dense aggregations of floating weeds)

Flowering aquatic plants are a serious problem in shallow, stagnant, or slow-flowing water in many tropical countries. They are expensive liabilities in newly impounded reservoirs in developing nations.

G Biological Pollution

Contamination of living native biotas by introduction of exotic life forms has been called biological pollution by Lachner et al. Some of these introductions are compared to contamination as severe as a dangerous chemical release. They also threaten to replace known wildlife resources with species of little or unknown value.

1 Tropical areas have especially been vulnerable. Florida is referred to as "a biological cesspool of introduced life".

2 Invertebrates

a Asian Clams have a pelagic veliger larvae, thus, a variety of hydro installations are vulnerable to subsequent pipe clogging by the adult clams.

b Melanian snails are intermediate hosts for various trematodes parasitic on man.

3 Vertebrates

a At least 25 exotic species of fish have been established in North America.

b Birds, including starlings and cattle egrets.

c Mammals, including nutria.

4 Aquatic plants

Over twenty common exotic species are growing wild in the United States. The problem of waterway clogging has been especially severe in parts of the Southeast.

III In polluted environments, there is a more noticeable change in balance, time response, and effects.

A Organic Pollution

The conditions here are classic and well described. There is a succession of biological communities (Figure 2) each of which modify the environment and water properties, thus, the effects are predominately biological.

1 Tubificidae (aquatic earthworms) in the first zone may reach as many as $1,7000,000/m^2$ and move up to 50 tons of mud per acre per day.

2 In the second zone chironomids (midge larvae) are found in thousands per square meter and may reduce the DO level by one and one-half ppm per stream mile.

3 The Isopod (sowbug) zone (the genus Asellus or Lirceus depending on locality) third in succession also may reach a density of thousands per square meter, a further oxygen demand due to respiration. (Figure 2)

4 The filamentous green alga Cladophora in both streams and lakes responds to organic enrichment by producing dense growths. (Figure 3)

5 Higher aquatic plants such as Potamogeton pectinatus may also be involved in these ecological changes in streams, particularly respiration vs. photosynthesis. There is correlation between weed bed growths, velocity and silt deposition. In some streams these massive growths on sloughing off foul water intakes and reduce DO levels as they decompose.

6 Sphaerotilus and/or slime growths below organic wastes, by metabolic demands while living and decomposition after death, impose a high BOD load on the stream and can severely deplete the dissolved oxygen. (Figure 3)

7 Blackflies (Diptera, family Simuliidae) often reach large populations below organic waste sources, filter feed on this material and in so doing, further degrade stream conditions with their fecal deposits.

B Inorganic and Toxic Pollution

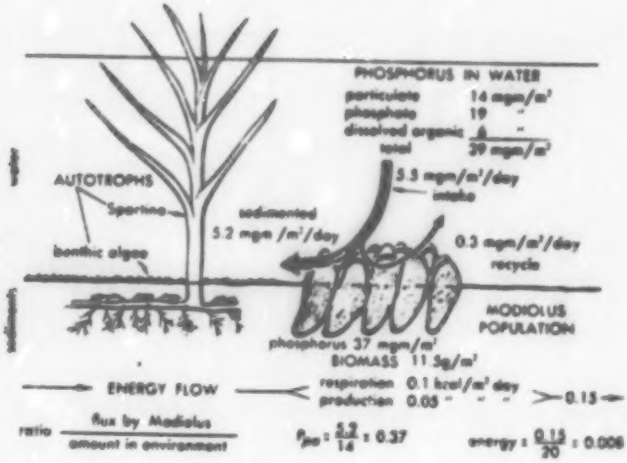
When progressive changes occur they are rarely produced by the biological community.

C Biological Magnification

Biological magnification is an additional chronic effect of toxic and other pollutants (such as heavy metals, pesticides, carcinogens, teratogens, radionuclides, bacteria, and viruses) which must be recognized and examined before clearance can be given for the disposal of a waste product into natural waters. To paraphrase Odum we could give nature an apparently innocuous amount of pollutant and have her give it back to us in a lethal or detrimental package.

1 Many animals, and especially bivalves, have the ability to remove from the environment and store in their tissues substances present at nontoxic levels in the surrounding water.

a This process may continue in the clam or fish, for example, until the body burden of the toxicant reaches such levels that the animal's death would result if the pollutant were released into the bloodstream by physiological activity.


PHOSPHORUS IN WATER
particulate 14 mgm/m²
phosphate 19 "
dissolved organic 6 "
total 39 mgm/m²
5.5 mgm/m²/day
intake
AUTOTROPHS
Spartina
benthic algae
sedimented
5.2 mgm/m²/day
0.3 mgm/m²/day
recycle
MODIOLUS
POPULATION
phosphorus 37 mgm/m²
BIOMASS 11.5g/m²
ENERGY FLOW
respiration 0.1 kcal/m² day
production 0.05 " " "
0.15
ratio flux by Modiolus / amount in environment
$P_{po} = \frac{5.2}{14} = 0.37$
energy = $\frac{0.15}{20}$ = 0.008

(Reproduced from Figure 4-4, from Ecology by Eugene P. Odum, copyright (c) 1963 by Holt, Rinehart and Winston, Inc. used by permission of Holt, Rinehart and Winston, Inc.)

Figure 1. The role of a shellfish (mussel) population in the cycling and retention of phosphorus in an estuarine ecosystem.

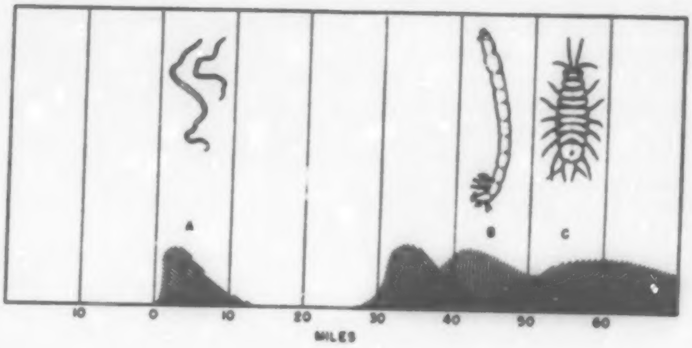


Figure 2. Linear alterations in populations of Tubificids (A) Chironomids (B), and Isopods (C) (from Bartsch). Zone C is often referred to as the Cladophora-Asellus Zone.

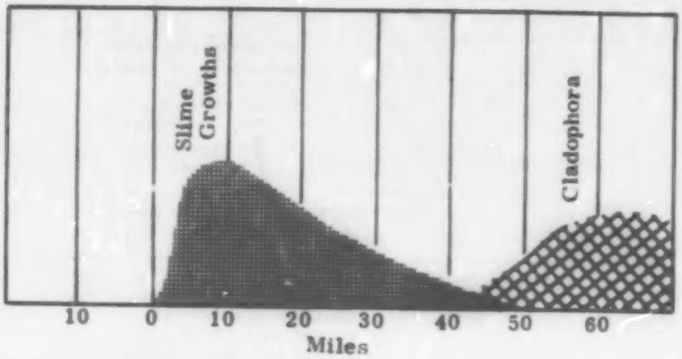


Figure 3. Linear alterations in populations of slime growths and Cladophora (modified from Bartsch)

b This may occur, as in the case of chlorinated hydrocarbon pesticides (such as DDT and endrin) stored in fat depots, when the animals food supply is restricted and the body fat is mobilized.

c The appearance of the toxicant in the bloodstream causes the death of the animal.

2 The biological magnification and storage of toxic residues of polluting substances and microorganisms may have another serious after effect.

a Herbivorous and carnivorous fish at lower trophic stages may gradually build up DDT residues of 15 to 20 mg/l without apparent ill effect.

b Carnivorous fish, mammals, and birds preying on these contaminated fish may be killed immediately or suffer irreparable damage because of the pesticide residue or infectious agent.

c Commercial shellfisheries are damaged by toxins produced by some dino-flagellate blooms in nearshore waters.

IV In summary, the biological causes of DO changes, associated changes in pH and CO_2, ammonia, nitrates, and sulphides, require study if the effects of organic pollution are to be calculated and predicted. Further biological effects on pollution include the relation to fouling organisms; stabilization of sediments in estuaries; recycling of nutrients; and problems of biological magnification.

REFERENCES

1 Curtis, E.J. Review Paper. Sewage Fungus: Its Nature and Effects. Water Res. 3:289-311. 1969.

2 Herbst, Richard P. Ecological Factors and the Distribution of Cladophora glomerata in the Great Lakes. Amer. Mid. Nat. 82(1):90-98. 1969.

3 Holm, L.G., Weldon, L.W. and Blackburn, R.D. Aquatic Weeds. Science. 66:699-709. 1969.

4 Odum, E.P. Ecology. Holt, Rinehart, and Winston. 192 pp. 1961.

5 Lachner, Ernest A., Robins, C. Richard, and Courtenay, Walter R. Jr. Exotic Fishes and Other Aquatic Organisms Introduced into North America Smithsonian Contrib. to Zool. 59:1-29. 1970.

6 Patrick, Ruth. Water Research Programs Aquatic Communities. Office Water Resources, U.S. Department of the Interior, Washington, D.C. 22 pp. 1968.

7 Sculthorpe, C.D. The Biology of Aquatic Vascular Plants. St. Martin's Press, New York. 610 pp. 1967.

8 Sebestyén, Olga. On Urnatella gracilis Leidy (Kamptozoa Cori) and its occurrence in an industrial waterworks fed by Danube water in Hungary. Acta Zool. Acad. Sci. Hungaricae. 8:435-448.

9 Westlake, D.F. The Effects of Organisms on Pollution. Proc. Linnean Soc. London. 170 session pt. 2. p. 171-172. 1959.

10 Westlake, D.F. The Effects of Biological Communities on Conditions in Polluted Streams. Symp. No. 8 Inst. of Biol. London (41 Queen's Gate, London, S.W.T.) p. 25-31. 1959.

11 Whitton, B.A. Review Paper. Biology of Cladophora in Freshwaters, Water Research 4:457-476. 1970.

This outline was prepared by Ralph M. Sinclair, Aquatic Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

EFFECTS AND CONTROL OF OIL POLLUTION

I INTRODUCTION

The increased size of oil tankers, density of waterborne traffic, and offshore petroleum production operations point up the need for prevention and control of oil spillage. For example, the Torrey Canyon wreck dumped upwards of 30 million gallons of oil on the coast of England in 1967; the Ocean Eagle wreck spread over three million gallons in San Juan Harbor alone. Other major spills also occurred in the York River, Virginia, and on the Cape Cod National seashore in 1967, and the well-known Santa Barbara, California, oil well incident contaminated over 400 miles of coastline.

A Specific Areas of Interest

1 On-scene control of gross leakage

2 Destruction or recovery of open sea or open water oil slicks

3 Disposal of recovered mixtures

4 Protection of the shore face and estuaries

5 Cleaning of shore face and estuaries

6 Effect of oil pollution and treatment agents on marine and freshwater flora and fauna

7 Waterfowl recovery methods

B Floating Sorbers and Sinkants

1 Examples of collecting agents

a Floating sorbers such as straw, or mineral perlites

b Plastic or other polymeric materials such as polyurethane foam

c Gelling agents

2 Floating Sorbers

a Inexpensive

b Can be disposed of by burning or burial

c Oil recovery very difficult

3 Plastics (burning not recommended)

a Expensive

b Excellent solution to problem since no residues are left on the bottom

c Large quantities of oil can be reclaimed for subsequent use

4 Gelling Agents

a In developmental stage

b May prove helpful in collecting spilled oil

5 Sinkants

a Sink oil, such as by certain chalks or carbonized sand

b Demersal fish species may be affected adversely

c Resurfacing of oil mass generally is probable, although it is delayed and slow

d Method should be applied only beyond continental shelf and in areas not involving commercial fisheries

6 The spreading of sorbers, polymeric materials, gelling agents, etc., is aided when spread by specialized equipment.

7 Confined slicks are easier to handle

C Chemical Treatment

1 A large number of dispersants and emulsifiers exist, although little quantitative or comparative information on toxicities and effectiveness to aquatic life is available.

2 The aromatic oil solvents used in the compounding of many of these agents have been found to be the principal toxic agent.

3 Based on review of the Torrey Canyon incident, opinion is divided on the general use of oil emulsifiers when water pollution control is of prime consideration. *

D Biological Degradation of Crude Oil and Oil Fractions in the Ocean and Fresh Water

1 Biological degradation is controlled by the following environmental conditions:

  a Nutrients

  b Temperature

  c O_2 availability

  d Degree of dispersion of oil

  e Species of bacteria

  f Other species of invertebrates

2 Degradation rates are very slow.

3 Seeding of oil slicks with microorganisms which feed specifically on the oil requires further research.

E Booming

1 The ability to confine a spill is principally a function of time, availability and efficiency of equipment, and prevailing environmental conditions.

2 If oil release occurs over several days, the ability to confine the oil to the immediate area would depend largely on the prevailing water conditions.

  a Booming in waters with a sea state greater than three is impractical with most of the presently existing boom designs.

  b Even with a sea state of three or less, this is suspect if wind conditions are adverse.

  c Currents in excess of 1.5 to 2 knots make booming difficult without extensive skirts and anchoring systems.

3 There are two principal types of mechanical barriers applicable to oil spills--floating booms and underwater bubble barriers.

  a Both presently are suitable only for calm water and are subject to failure.

  b The floating boom is generally superior to the bubble barrier on an emergency basis as it is more portable.

  c The main advantage of a bubble barrier is the unrestricted entry and exit of ships.

---

*Review Dept. of Interior policy on use of chemicals to treat oil (in lack of national contingency plan) available as handout, or from Dept. of Interior.

d The main disadvantage is the complete loss of containment in the event of air supply failure.

F Burning

1 If all attempts to salvage a vessel or cargo have failed and a ship has been abandoned, an attempt should be made to set the oil afire while still contained in the vessel.

2 Burning on the surface generally is not effective due to the rapid transfer of heat to the water, selective burning of lighter-fractions, and lack of O_2 supply except at the edge of a slick.

3 Caution should be exercised in the vicinity of ships, docks, etc.

G Skimming

1 Mechanical devices for collecting oil from the surface of water, such as rotating cylinders and suction pumps, generally are available but have relatively small capacities.

2 The use of these devices is restricted to relatively calm waters if reasonable efficiency is to be achieved.

This outline was prepared by John Wooley, Formerly Aquatic Biologist, Pacific Northwest Water Laboratory, WQO, EPA, Corvallis, OR 97330.

SECTION C

BIOLOGICAL TECHNIQUES IN WATER QUALITY INVESTIGATIONS

Outline Number

Techniques for the Biological Evaluation of Pollutants C- 1

Field Investigations

Biological Field Methods C- 2

The Identification of Organisms

Critical Problems in Systematics C- 3

The System of Biological Classification C- 4

Plankton Studies

Techniques of Plankton Analysis C- 5

Determination of Plankton Productivity C- 6

Artificial Substrates

Artificial Substrates C- 7

Artificial and Related Substrates - References C- 8

Fish Kills

Procedures for Fish Kill Investigations C- 9

Human Health

Bacteriological Indicators of Water Pollution C-10

Evaluation of Toxicity

Bioassay and Biomonitoring C-11

Bottom Organisms

Using Benthic Biota in Water Quality Evaluations C-12

Periphyton

Attached Growths (Periphyton or Aufwuchs) C-13

Stream Invertebrate Drift C-14

TECHNIQUES FOR THE BIOLOGICAL EVALUATION OF POLLUTANTS

I INTRODUCTION

A Ways in Which Wastes Affect Aquatic Life

1 Indirect: by modification of the environment such as by modifying food chain, changing average annual temperature, or reducing DO.

2 Direct: physical or physiological action on the organism itself.

3 Rendering aquatic product distasteful or dangerous for human consumption.

B Two Basic Approaches: field observation, or laboratory tests.

NOTE: Equipment and procedures for making the following determinations and interpretations of results, will not be considered.

II FIELD OBSERVATIONS

A Field evaluations are usually based on comparisons with an actual or imaginary unpolluted reference or "control" site. For example, what are the actual conditions:

1 In a stream below a point of pollution as compared to above (an actual or real control).

2 In any polluted area compared to "what it might, or should be" (this is a theoretical control, depends on the skill and integrity of the evaluator for its usefulness).

3 In any polluted area, what are conditions now as compared to what they were before pollution was introduced? This assumes that a pre-pollution study or observation was made, which in this case constitutes the control.

Field controls in general are much less rigorous than laboratory controls. Liberal margins of safety must thus be provided when extrapolating laboratory results to field conditions.

B Two general types of biological evaluations may be employed in the above comparisons: qualitative (descriptive) and quantitative (measurement).

1 Qualitative observation

a The indicator concept is generally based on the positive thought that there must be some organism which is found in polluted areas but not in unpolluted areas.

1) This is true only for the bacteria where certain forms present in the intestines of warm-blooded animals can be found in sewage. Finding and identifying these organisms thus demonstrates a strong likelihood that animal excrement is present.

2) Higher forms of life do not usually live in the sources of pollution, especially industrial, and hence are not carried thence into the receiving water. There is no known form of pollution-tolerant higher life which cannot also be found in unpolluted places. The finding of a pollution-tolerant organism does not therefore necessarily demonstrate pollution. There is, however, infinite variety to the sensitivity of various forms of higher life to the various forms of pollution.

b Population balance, that is, the species composition of the aquatic community, is very sensitive to environmental conditions, and hence to pollution.

2 Quantitative observation and measurements

a Quantitative data may have reference to the entire aquatic community, or only to selected or individual species. Reference may be to numbers or to weight. Weight may be wet, dry, ashed, etc. Rate of production may be involved. Much remains to be done in this field.

b Productivity is the ability of an environment to produce or grow a crop of organisms (corn, hogs, algae, fish, mayflies). Productivity may be measured in some stipulated unit of quantity per unit of volume or area per unit of time. For example:

- Grams of carbon fixed (by photosynthesis) per square meter per day;
- Pounds of fish produced per acre per year;
- Number of fish catchable per fisherman-hour.

c The standing crop is an estimate of the quantity (or biomass) of some specified portion of the aquatic community present at a point in time. For example:

- Pounds of large mouthed black bass per acre;
- Grams of pollution-intolerant bottom invertebrates per square foot, square meter, etc.;
- Total plankton count per ml, or per liter
- Total quantity of all life present per unit area, volume, etc.

3 Various workers have developed formulas for expressing the degree or nature of pollution based on one or more of the above determinations. While valid and useful in the hands of the originator and his staff, none has yet been universally accepted.

III LABORATORY EVALUATIONS

These, too, almost invariably involve a comparison or "control" setup. Such studies may have broad and general objectives, or be designed to detect life history stages exhibiting maximum sensitivity such as eggs, juveniles, breeding condition, etc.

A Bioassays are assays of the biological effect of something, employing living organisms as the yardstick. They are widely employed in commercial, scientific, and water quality surveillance activities.

1 Methods useful for aquatic organisms are described in Standard Methods. Test organisms are exposed to a series of concentrations of some substance for a stated period of time under stated conditions.

a If the material is highly toxic in the concentrations employed and the organisms in the higher concentrations die, "tolerance limits" can be calculated. A common parameter employed is the concentration which 50% of the organisms can tolerate (or survive) for time "t" called the "median tolerance limit" (for time "t"). This may be written "TL_m" 96hrs. for the 96 hour median tolerance limit, for example.

  - b Any appropriate organism may be employed, from protozoa to fish.

2 "Static jars" or "continuous flow" apparatus may be employed to provide the various dilutions of the toxicant being tested.

  - a Static jar tests are seldom run for more than a week, and are usually read only in terms of percent survival or kill, generally termed "acute" toxicity.
  - b Continuous flow apparatus may likewise be employed to measure short term acute toxicity, but it is virtually essential for long term tests at sublethal concentrations where parameters other than lethal thresholds, at given time intervals, are measured; e.g., tests of a series of sublethal concentrations of a toxicant on the growth rate and breeding success of a species of fish, lasting for one or two years.

B A recently published procedure called biomonitoring provides for the continuous surveillance of an industrial effluent (or a dilution thereof) using live organisms as the test of its suitability for discharge.

C A conventional BOD determination is a bioassay of biologically oxidizable material present.

D Combination laboratory and field studies are often conducted to determine the effects of specific substances or conditions, often on specific organisms; for example, the effect of heated water discharges on oysters, or the effect of endrin on catfishes.

E Post mortem examination of dead organisms, especially fishes, can occasionally shed light on the cause of death. For example, the ratio of zinc in the gill structure to zinc in the opercular bone can indicate death caused by a sudden increase in the zinc concentration of the water. Unfortunately, relatively few substances have been so analyzed.

REFERENCES

1 Hutcheson, George E. A Treatise on Limnology. John Wiley Company, New York. 1957.

2 Jackson, H.W., and Brungs, W.A. Biomonitoring of Industrial Wastes. Purdue Industrial Waste Conference, West Lafayette, Indiana. May 3-5, 1966.

3 Mount, D.I. An Autopsy Technique for Zinc-Caused Fish Mortality. Trans. Am. Fish Soc. 93 (2):174-182. April 1964.

4 Standard Methods for the Examination of Water and Wastewater. 12th Ed. APHA, AWWA, WPFC. Published by Am. Pub. Health Assoc., New York. 1965.

5 Welch, P.S. Limnological Methods. Blakiston Co., Philadelphia, Pa. 1948.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Environmental Protection Agency, OWP, Cincinnati, OH 45268

BIOLOGICAL FIELD METHODS

I INTRODUCTION

A Due to the nature of ecological interrelationships, methods for the collection of different types of aquatic organisms differ. In general we can recognize those that swim or float and those that crawl, those that are big and those that are little. Each comprises a part of "the life" at any given survey station and consequently a "complete" collection would include all types.

B Field methods in the following outline are grouped under four general categories, the collection of:

1 Benthos (or bottom dwelling organisms). These may be attached, crawling, or burrowing forms.

2 Plankton (plancton). These are all of the microscopic plants and animals normally swimming or suspended in the open water.

3 Periphyton or "aufwuchs". This is the community of organisms associated with the surfaces of objects. Some are attached, some crawl. The group is intermediate between the benthos and the plankton.

4 Nekton. Nekton are the larger, free swimming active animals such as shrimp or fishes.

C Aquatic mammals and birds, in most cases, require still other approaches and are not included.

D There is little basic difference between biological methods for oceanic, estuarine, or freshwater situations except those dictated by the physical nature of the environments and the relative sizes of the organisms. Fish, benthos, and plankton collection is essentially the same whether conducted in Lake Michigan, Jones' Beach, or the Sargasso Sea.

1 Marine organisms range to larger sizes, and the corrosive nature of seawater dictates special care in the design and maintenance of marine equipment. Site selection and collection schedules are influenced by such factors as tidal currents and periodicity, and salinity distribution, rather than ,(river) currents, riffles, and pools.

2 Freshwater organisms are in general smaller, and the water is seldom chemically corrosive on equipment. Site selection in streams involves riffles, falls, pools, etc., and a unidirectional flow pattern. Lake collection may involve less predictable stratification or flow patterns.

E Definite objectives should be established in advance as to the size range of organisms to be collected and counted, i.e.: microscopic only, microscopic and macroscopic, those retained by "30 mesh" screens, invertebrates and/or vertebrates, etc.

II STANDARD PROCEDURES

A Certain standard supplementary procedures are a part of all field techniques. In order to be interpreted and used, every collection must be associated with a record of environmental conditions at the time of collection.

1 Data recorded should include the following as far as practicable.

Location (name of river, lake, etc.)

Station number (particular location of which a full description should be on record)

Date and hour

Air temperature

Water temperature (at various depths, if applicable)

Salinity (at various depths, if applicable)

Tidal flow (ebb or flood)

Turbidity (or light penetration, etc.)

Weather

Wind direction and velocity

Sky or cloud cover

Water color

Depth

Type of bottom

Type of collecting device and accessories

Method of collecting

Type of sample (quantitative or qualitative)

Number of samples at each station

Chemical and physical data, e.g., dissolved oxygen, nutrients, pH, etc.

Collector's name

Miscellaneous observations (often very important)

2 All collecting containers should be identified at least with location, station number, sample number, and date. Spares are very handy.

3 Much transcription of data can be eliminated by using sheets or cards with a uniform arrangement for ,including the above data. The same field data sheet may include field or laboratory analysis.

B Compact kits of field collecting equipment and materials greatly increase collecting efficiency, especially if collection site is remote from transportation.

III PERSONAL OBSERVATION AND PHOTOGRAPHY

A Direct or indirect observation of underwater conditions has become relatively efficient.

1 Diving spheres, pioneered by William Beebe, Cousteau, Honot, Willm, and Manad are proving very important for deep water observations.

2 Use of the aqualung permits direct personal study down to over 200 feet.

3 Underwater television (introduced by the British Admiralty for military purposes) is now generally available for biological and other observations.

4 Underwater photography is improving in quality and facility.

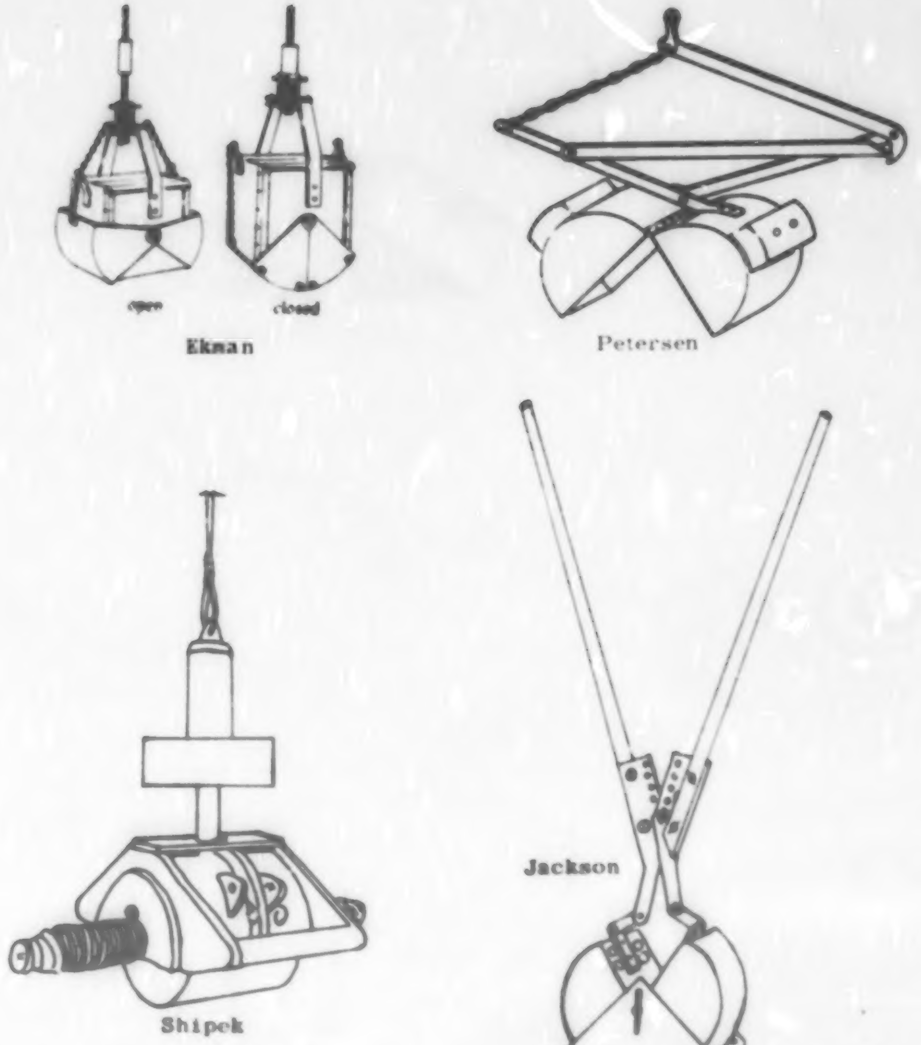
5 Underwater swimming or use of SCUBA is quite valuable for direct observation and collecting.

IV COLLECTION OF BOTTOM OR BENTHIC ORGANISMS

A Shoreline or Wading Depth Collecting Plates I, II

1 Hand picking of small forms attached to or crawling on rocks, sticks, etc. when lifted out of the

BOTTOM GRABS


open
closed

Ekman

Petersen

Shipek

Jackson

PLATE I

LIMNOLOGICAL EQUIPMENT

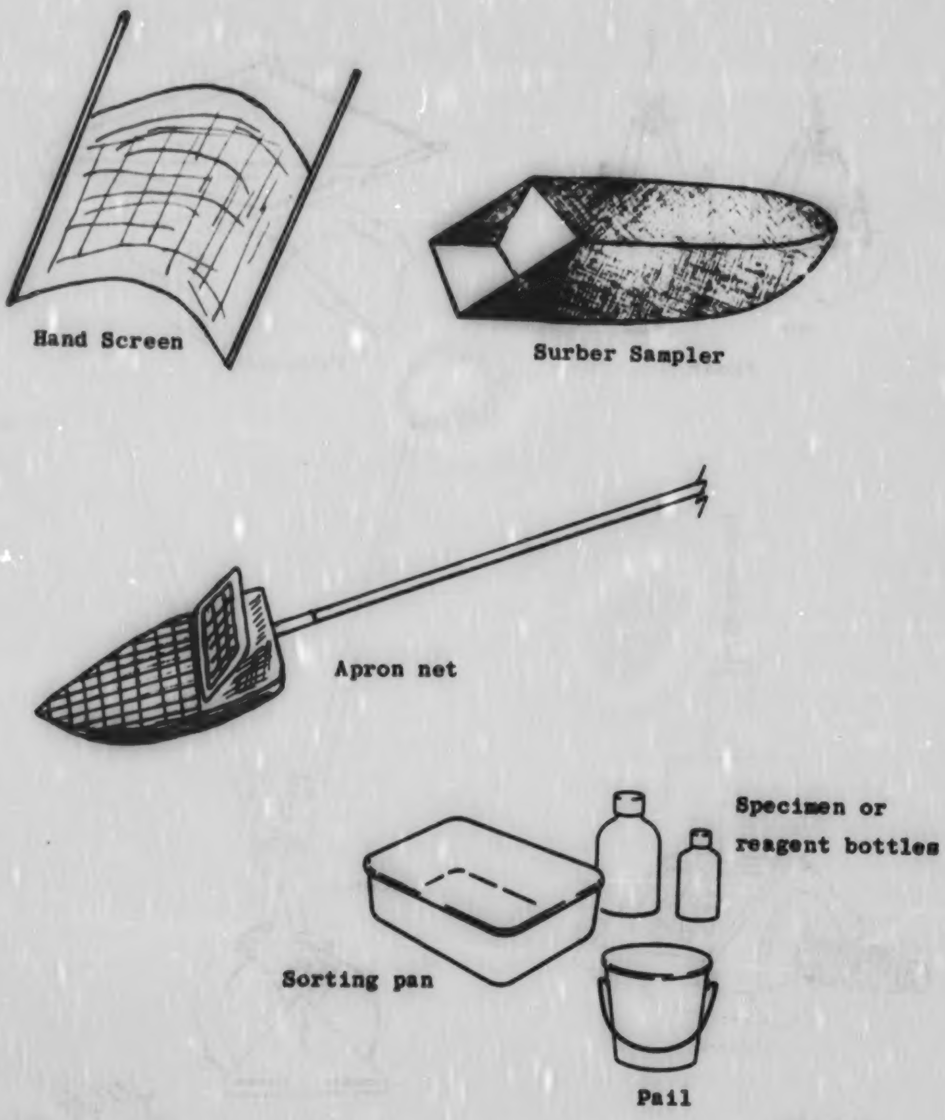

Hand Screen
Surber Sampler
Apron net
Specimen or
reagent bottles
Sorting pan
Pail

PLATE II

water is a fundamental and much used method for quickly assaying what is present and what may be expected on further search.

2 Patches of seaweed and eelgrass and shallow weedy margins anywhere are usually studied on a qualitative basis only.

a The apron net is one of the best tools for animals in weed beds or other heavy vegetation. It is essentially a pointed wire sieve on a long handle with coarse screening over the top to keep out leaves and sticks.

b Grapple hooks or a rake may be used to pull masses of vegetation out on the bank where the fauna may be examined and collected as they crawl out.

c Quantitative estimates of both plants and animals can be made with a "stove pipe" sampler which is forced down through a weed mass in shallow water and embedded in the bottom. Entire contents can then be bailed out into a sieve and sorted.

d A frame of known dimensions may be placed over an area to be sampled and the material within cropped out. This is especially good for larger plants and large bivalves. This method yields quantitative data.

3 Sand and mud flats in estuaries and shallow lakes may be sampled quantitatively by marking off a desired area and either digging away surrounding material or excavating the desired material to a measured depth. Handle-operated samplers recently developed by Jackson and Larrimore, make for more effective sampling of a variety of bottoms down to the depth of the handles. Such samples are then washed through graded screens to retrieve the organisms.

4 Ekman grabs are most useful on soft bottoms. This is a completely closing clamshell type grab with spring operated jaws. Size of grab is usually 6" × 6" or 9" × 9", the 12" × 12" size is impractical due to its heavy weight when filled with bottom material.

For use in shallow water, it is convenient to rig an Ekman with a handle and a hand operated jaw-release mechanism.

5 The Petersen type grab (described below) without weights will take satisfactory samples in firm muds, but tends to bury itself in very soft bottoms. It is seldom used in shallow water except as noted below.

B Collecting in Freshwater Riffles or Rapids

1 The riffle is one of the most satisfactory habitats for comparing stream conditions at different points.

2 The hand screen is the simplest and easiest device to use in this situation. Resulting collections are qualitative only.

a In use the screen is firmly planted in the stream bed. Upstream bottom is thoroughly disturbed with the feet, or worked over by hand by another person. Organisms dislodged are carried down into the screen.

b Screen is then lifted and dumped into sorting tray or collecting jar.

3 The well-known square foot Surber sampler is one of the best quantitative collecting devices for riffles.

a It consists of a frame one foot square with a conical net attached. It is usable only in moving water.

b In use it is firmly planted on the bottom. The bottom stones and gravel within the square frame are then carefully gone over by hand to ensure that all organisms have been dislodged and carried by the current into the net. A stiff vegetable brush is often useful in this regard.

c From three to five square-foot samples should be taken at each station to insure that a reasonable percentage of the species present will be represented.

4 The Petersen type grab may be used in deep swift riffles or where the Surber is unsuitable.

a It is planted by hand on the bottom, and worked down into the bottom with the feet.

b It is then closed and lifted by pulling on the rope in the usual manner.

5 A strong medium weight dipnet is the closest approach to a universal collecting tool (effective for everything from Blondes to indicator organisms!)

a This is used with a sweeping motion, through weeds, over the bottoms or in open water. A triangular shape is preferred by some. This may be used as a roughly quantitative device in riffles by holding the end flat against the bottom and backing slowly up-stream disturbing the substrate with one's feet. A standard period of time is used.

b The handle should be from 4 to 6 feet long, and about the weight of a garden rake handle.

c The ring should be made of steel or spring brass, and securely fastened to the handle. It should be strong but not cumbersome; size of ring stock will depend on diameter of ring.

d The bag or net should be the strongest available, not over 1/8 inch mesh, preferably about 1/16 inch. Avoid 30 or more meshes to the inch; this is so fine that the net plugs too easily and is slow and heavy to handle.

e There should be a wide canvas apron sewed around the rim and protecting the bag. The rim may be protected with leather if desired.

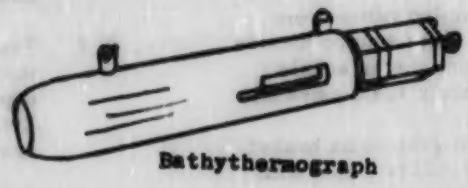
D Deep Water Benthic Collecting Plate III

1 When sampling from vessels, a crane and winch, either hand or power operated, is used. The general ideas described for shallow waters apply also to deeper waters, when practicable.

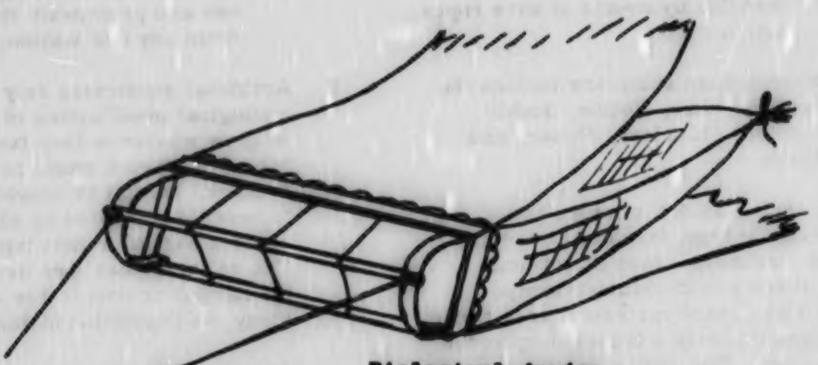
2 The Petersen type grab, seems to be the best all around sampler for the greatest variety of bottoms at all depths, from shoreline down to over 10,000 meters. (Plate I)

a It consists of two heavily constructed half cylinders closed together by a strong lever action.

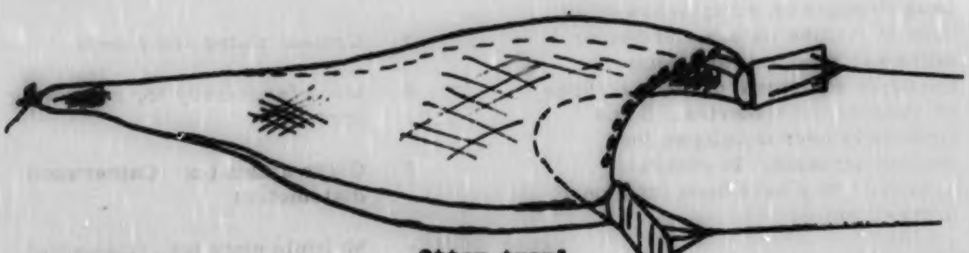
DEEP WATER EQUIPMENT



Bathythermograph



Biological dredge



Otter trawl

PLATE III

b To enable them to bite into hard bottoms, or to be used in strong currents, weights may be attached to bring the total weight up to between 50 and 100 lbs.

c Areas sampled range from 1/5th to 1/20th square meters (1/10 square meter equals approximately 1.1 square ft.)

d A Petersen grab to be hauled by hand should be fitted with 5/8 or 3/4 inch diameter twisted rope in order to provide adequate hand grip. It is best handled by means of wire ropes and a winch.

3 Other bottom samplers include the VanVeen, Lee, Holme, Smith-McIntyre, Knudsen, Ponar, and others.

4 A spring loaded sampler has recently been developed by Shipek for use on all types of bottoms. It takes a half-cylinder sample, 1/25th square meters in area and approximately 4 inches deep at the center. The device is automatically triggered on contact with the bottom, and the sample is completely protected enroute to the surface. (Plate I)

5 Drag dredges or scrapes are often used in marine waters and deeper lakes and streams, and comprise the basic equipment of several types of commercial fisheries. Some types have been developed for shallow streams. In general however, they have been little used in fresh water.

6 The above is only a partial listing of the many sampling devices available. Others that are often encountered are the orange-peel bucket, plow dredge, scallop type dredge, hydraulic dredges, and various coring devices. Each has its own advantages and disadvantages and it is up to the worker and his operation to decide what is best for his particular needs. The Petersen type and Ekman grabs are perhaps the most commonly used.

7 Traps of many types are used for various benthic organisms, especially crabs and lobsters. Artificial substrates (below) are in essence a type of trap.

8 Since most biological communities are not evenly distributed, it is advisable to routinely take at least two and preferably more samples from any one station.

E Artificial substrates rely on the ecological predilection of organisms to grow wherever they find a suitable habitat. When a small portion of artificial habitat is provided, it tends to become populated by all available species partial to that type of situation. The collector can then at will remove the habitat or trap to his laboratory and study the population at leisure.

This versatile research technique is much used for both routine monitoring and exploratory studies of pollution. It is also exploited commercially, especially for shellfish production. Types of materials used include:

1 Cement plates and panels.

2 Wood (especially for burrowing forms).

3 Glass slides (ex: Catherwood diatometer).

4 Multiple plate trap (masonite).

5 Baskets (or other containers) holding natural bottom material and either imbedded in the bottom, or suspended in the overlying water.

6 Unadorned ropes suspended in the water, or sticks thrust into the bottom.

F Sorting and Preservation of Collections

1 Benthic collections usually consist of a great mass of mud and other debris among which the organisms are hidden. Various procedures may be followed to separate the organisms.

a The organisms may be picked out on the spot by hand or the entire mess taken into the laboratory where it can be examined more efficiently (especially in rough weather). Roughly equivalent time will probably be required in either case.

b Specimens may be simply observed and recorded or they may be preserved as a permanent record.

c Organisms may be simply counted, weighed, or measured volumetrically; or they may be separated and recorded in groups or species.

2 If separation is in the field, this is usually done by hand picking, screening, or some type of flotation process.

a Hand picking is best done on a white enameled tray using light touch limnological forceps.

b Screening is one of the most practical methods to separate organisms from debris in the field. Some prefer to use a single fine screen, others prefer a series of 2 or 3 screens of graded sizes. The collection may be dumped directly on the screen and the mud and debris washed through, or it may be dumped into a bucket or small tub. Water is then added, the mixture is well stirred, and the supernatant poured through the screen. The residue is then examined for heavy forms that will not float up.

c A variation of this method in situations where there is no mud is to pour a strong sugar or salt solution over the collection in the bucket, stir it well, and again pour the supernatant through the screen. This time, however, saving the flotation solution for re-use. The heavier-than-water solution accentuates the separation of organisms from the debris (except for the heavy shelled molluscs, etc.). A solution of 2-1/2 lbs. of sugar per gallon of water is considered to be optimum.

3 Preservation or stabilization is usually necessary in the field.

a 95% ethanol (ethyl alcohol) is highly satisfactory. A final strength of 70% is necessary for prolonged storage. If the collection is drained of water and flooded with 95% ethanol in the field, a laboratory flotation separation can usually be made later, thus saving much time. Considerable quantities of ethanol are required for this procedure.

b Formaldehyde is more widely available and is effective in concentrations of 3 - 10% of the commercial formulation. However, it shrinks and hardens specimens, collector, and laboratory analyst without favor! In order to minimize bad effects from formalin, neutralized formalin is recommended. Mollusc shells will eventually disintegrate in acid formalin

c Properly preserved benthos samples may be retained indefinitely, thereby enhancing their utility.

d Refrigeration or icing is very helpful.

V MICROFAUNA AND PERIPHYTON (OR AUFWUCHS) SAMPLING

A This is a relatively new area which promises to be of great importance. The microfauna of mud and sand bottoms may be studied to some extent from collections made with the various devices mentioned above. In most cases however, there is considerable loss of the smaller forms.

B Most special microfauna samplers for soft bottoms are essentially modified core samplers in which an effort is made to bring up an undisturbed portion of the bottom along with the immediately overlying water. The best type currently seems to be the Enequist sampler which weighs some 35 kg. and takes a 100 sq. cm sample 50 cm. deep.

C Microfauna from the surface of hard sand or gravel bottoms may be sampled by the Hunt vacuum sampler. This has a bell-shaped "sampling" tube sealed by glass diaphragm. On contact with the bottom, the glass is automatically broken and the nearly bottom material is swept up into a trap.

D Periphyton attached to or associated with hard surfaces such as rock or wood may be sampled by scraping or otherwise removing all surface material from a measured area. The periphyton, however, is more effectively quantitatively sampled by artificial substrate techniques described above.

VI THE COLLECTION, OR SAMPLING OF PLANKTON PLATE IV

A Phytoplankton: A Planned Program is Desirable

1 A planned program of plankton analysis should involve periodic sampling at weekly or even more frequent intervals.

2 A well-planned study or analysis of the growth pattern of plankton in one year will provide a basis for predicting conditions the following year since seasonal growth patterns tend to repeat themselves from year to year.

a Since the seasons and the years differ, records accumulated over the years become more useful.

b As the time for an anticipated bloom of some troublesome species approaches, the frequency of analyses may be increased.

3 Detection of a bloom in its early stages will facilitate more economical control.

B Field Aspects of the Analysis Program

1 Two general aspects of plankton analysis are commonly recognized: quantitative and qualitative.

a Qualitative examination tells what is present.

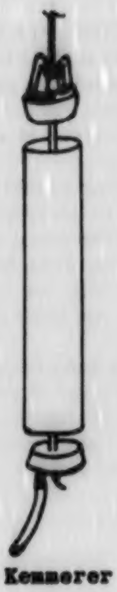
b Quantitative tells how much.

c Either approach is useful, a combination is best.

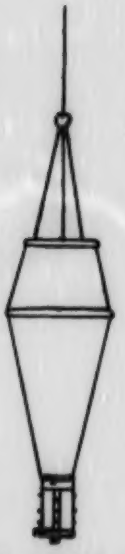
2 Equipment for collecting samples in the field is varied.

a A half-liter bottle will serve for surface samples of phytoplankton, if carefully taken.

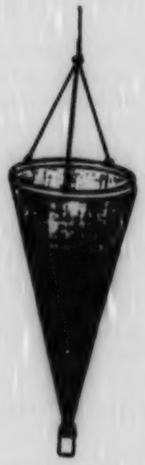
PLANKTON SAMPLERS

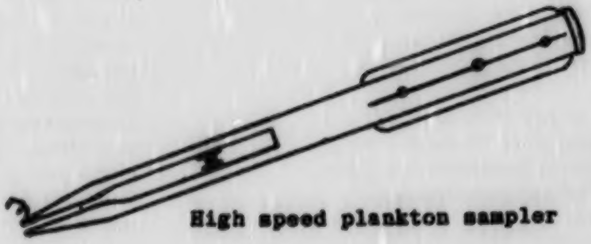


Kemmerer



Wisconsin net





High speed plankton sampler

PLATE IV

b A Kemmerer, Nansen, or other special sampler (small battery operated pumps are time saving) is suggested for depth samples.

c Plankton nets concentrate the sample in the act of collecting and also capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative however. For phytoplankton, #20 or #25 size nets are commonly used. Usually a net diameter of 5 - 10 inches is sufficient. The smaller forms however, are lost through any net.

C Zooplankton Collecting

1 Since zooplankton have the ability to swim away from water bottles, etc. nets towed at moderately fast speed are used for their capture. Number 12 nets (aperature size 0.119 mm, 125 meshes 1 inch) or smaller numbered net sizes are commonly used. A net diameter greater than 5" is preferred.

Frequently half meter nets or larger are employed. These may be equipped with flow measuring devices for measuring the amount of water entering the net.

2 Other instruments such as the Clark-Bumpus, Gulf-Stream, Hardy continuous plankton recorder, and high-speed instruments are used for collecting zooplankton, also.

3 The devices used for collecting plankton capture both the plant and animal types. The mesh size (net no.) is a method for selecting which category of plankton is to be collected.

D The Location of Sampling Points

1 Both shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 6 inches to one foot. The surface film is often significant.

b "Deep" samples should be taken at such intervals between surface and bottom as circumstances dictate. In general, the entire water column should be sampled as completely as practicable, and the plankton from each level recorded separately.

2 For estuarine plankton, it is necessary to sample different periods in the stage of the tide, otherwise samples would be biased to a given time, or type of water carried by the tidal currents.

3 Plankton is subjected to the force of the winds and currents. As a result, the plankton is often in patches or "wind rows". For this reason when using a net, it is often desirable to tow the net at right angles to the wind or current.

4 Not only are all plankton likely to be horizontally discontinuous, but zooplankton especially tend to be numerous near the bottom in daylight, but distributed more evenly through the water column at night. Therefore, a series of tows or samples at different depths is necessary to obtain a complete sampling. One technique often employed is to take an oblique tow from the bottom to the top of the water column.

5 Pilot studies to indicate sampling locations and intervals are often mandatory. Some studies require random sampling points.

6 The number of sampling stations that should be established is limited by the capability of the laboratory to analyze the samples, but should approach the needs of the objectives as closely as possible.

7 Field conditions greatly affect the plankton, and a record thereof should be carefully identified with the collection as in II above.

8 Provisions should be made for the field stabilization of the sample until the laboratory examination can be made if more than an hour or so is to elapse.

a Refrigeration or icing is very helpful, but ice should never be placed in the sample.

b Preservation by 5% formalin is widely used but badly shrinks animals and makes all forms brittle.

c Lugols solution is a good preservative.

d Ultra-violet sterilization is sometimes used in the laboratory to retard the decomposition of plankton.

e A highly satisfactory merthiolate preservative has been developed by the FWPCA and described by Weber (1968).

VII COLLECTING FISH AND OTHER NEKTON PLATES V, VI

A Fish and other nekton must be sought in the obscure and unlikely areas as well as the obvious locations in order for the collection to be complete. Several techniques should be employed where-ever possible (this is appropriate for all biota). It is advisable to check with local authorities to inform them of the reasons for sampling, because many of the techniques are not legal for the layman. In this area, perhaps more than any other, professionally trained workers are important. Also, there must be at least one helper, as a single individual always has difficulty in pulling both ends of a 20 foot seine simultaneously! The more common techniques are listed below.

B Seines

1 Straight seines range from 4-6 feet and upwards in length. "Common sense" minnow seines with approximately 1/4 inch mesh are widely used along shore for collecting the smaller fishes.

2 Bag seines have an extra trap or bag tied in the middle which helps trap and hold fish when seining in difficult situations.

C Gill nets are of use in offshore and/or deep waters. They range in length from approximately 30 yards upward. A mesh size is designed to catch a specified size of fish. The trammel net is a variation of the gill net.

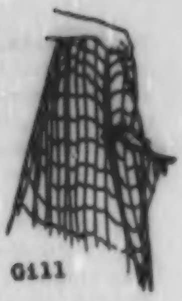
D Traps range from small wire boxes or cylinders with inverted cone entrances to semi-permanent weirs a half mile or more in length. All tend to induce fish to swim into an inner chamber protected by an inverted cone or V - shaped notch to prevent escape. Current operated rotating fish traps are also very effective (and equally illegal) in suitable situations.

E Trawls are submarine nets, usually of considerable size, towed by vessels at speeds sufficient to overtake and scoop in fish, etc. The mouth of the net must be held open by some device such as a long beam (beam trawl) or two or more vanes or "otter boards" (otter trawl). Plate III

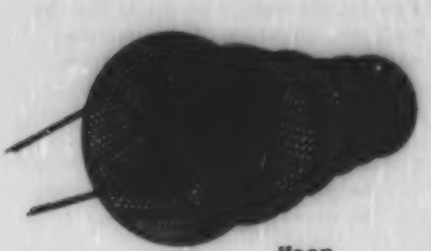
1 Beam and otter trawls are usually fished on the bottom, but otter trawls when suitably rigged are now being used to fish mid-depths.

2 The midwater trawl resembles a huge plankton net many feet in diameter. It is proving very effective for collecting at mid-depths.

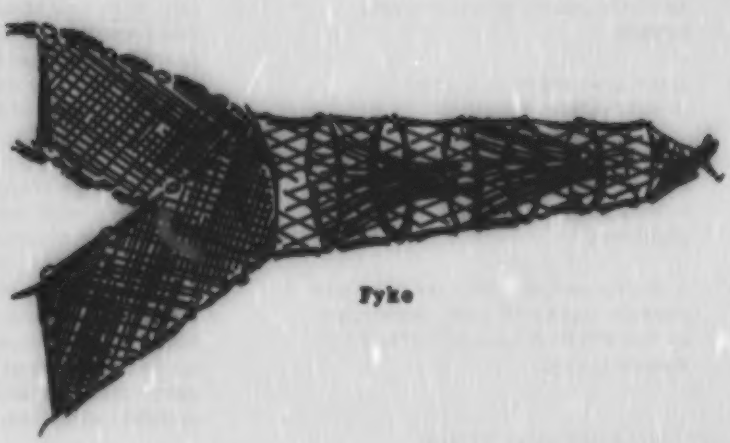
FISH NETS



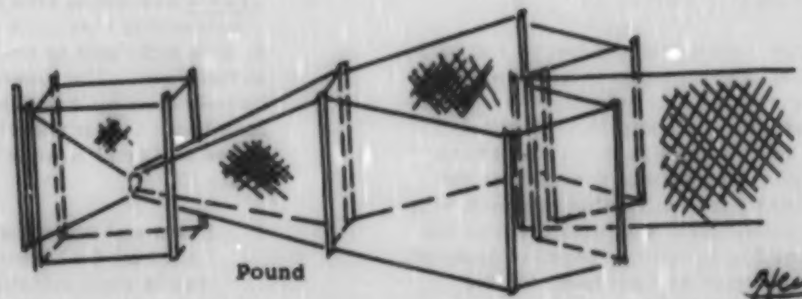
Gill



Hoop

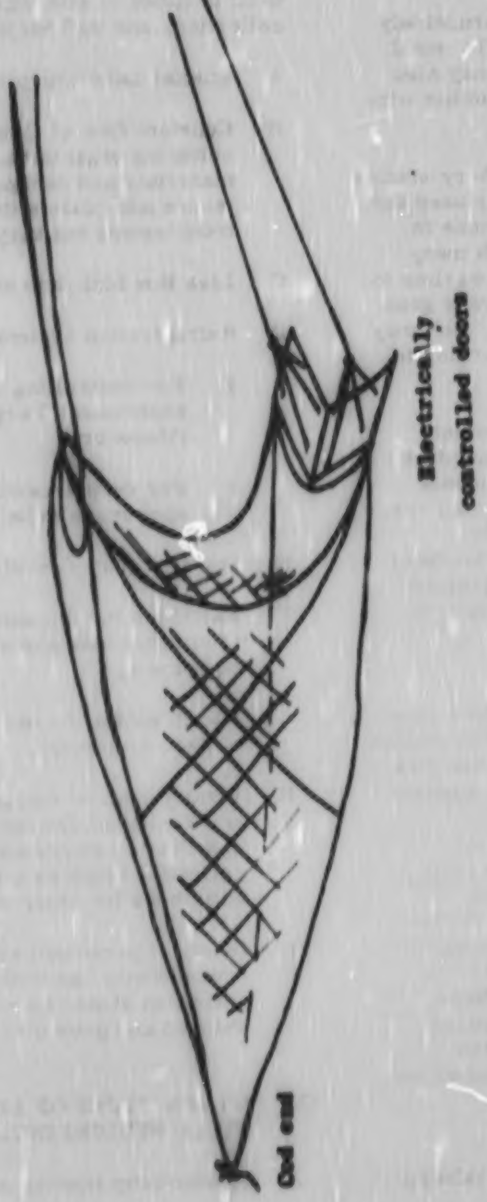


Fyke



Pound

PLATE V


Electrically controlled doors
Cod end

MIDWATER TRAWL

PLATE VI

PLATE VI

Numerous special designs have been developed. Plate VI

F Electric seines and screens are widely employed by fishery workers in small and difficult streams. They may also be used in shallow water like areas with certain reservations.

G Poisoning is much used in fishery studies and management. Most widely used and generally satisfactory is rotenone in varying formulations, although many others have been employed from time to time, and some appear to be very good. Under suitable circumstances, fish may even be killed selectively according to species.

H Personal observation by competent personnel, and also informal inquiries and discussions with local residents will often yield information of real use. Many laymen are keen observers, although they do not always understand what they are seeing. The organized creel census technique yields data on what and how many fish are being caught.

I Angling remains in its own right a very good technique in the hands of the skilled practitioner, for determining what fish are present. Spear-fishing also is now being used in some studies.

J Fish and other nekton are often tagged to trace their movements during migration and at other times. Miniature radio transmitters can now be attached or fed to fish (and other organisms) which enable them to be tracked over considerable distances. Physiological information is often obtained in this way. This is known as telemetry.

VIII SPECIAL REQUIREMENTS ON BOATS

Handling biological collections (as contrasted to chemical and physical sampling) on board boats differs with the size of the craft and the magnitude of operations. Some possible items are listed below. Hoisting and many other types of gear are used in common with other types of collection, and will not be listed.

A Special Laboratory Room(s)

B Constant flow of Clean water for culturing organisms. (Selection of materials and design of a system to insure non-toxic water may be very troublesome but very important.)

C Live Box built into ship at water level

D Refrigeration System(s)

  1 For controlling temperature of experimental organisms in laboratory.

  2 For deep-freezing and storage of specimens to be examined later.

E Storage Space (Unrefrigerated)

F Facilities for the safe storage and use of microscopes and other laboratory equipment.

G Facilities for the safe storage and use of deck equipment.

H Administrative access to the Captain and Technical Leader in order to coordinate requirements for biological collection (such as a slow plankton tow) with those for other collections.

I Safety of personnel working in and around boats, as well as in other field activities should be seriously considered and promoted at all times.

IX OTHER TYPES OF BIOLOGICAL FIELD STUDIES INCLUDE

A Productivity Studies of Many Types

B Life Cycle and Management

C Distribution of Sport or (potentially) Commercial Species

D Scattering Layers and Other Submarine Sound Studies

E Artificial Culture of Marine Food Crops

F Radioactive Uptake

G Growth of Surface-Fouling Organisms

H Marine Borers

I Dangerous Marine Organisms

J Red Tides

K Others

X SOURCES OF COLLECTING EQUIPMENT

Many specialized items of biological collecting equipment are not available from the usual laboratory supply houses. Consequently, the American Society of Limnology and Oceanography has compiled a list of companies handling such items and released it as "Special Publication No. 1, Sources of Limnological and Oceanographic Apparatus and Supplies." Available from the Secretary of the Society.

REFERENCES

1 Arnold, E.L., Jr. and Gehringer, J.W. High Speed Plankton Samplers, U.S. Fish and Wildlife Spec. Sci. Rept. Fish No. 88:1-6.

2 Barnes, H. (ed.). Symposium on New Advances in Underwater Observations. Brit. Assoc. Adv. Sci., Liverpool. pp. 49-64. 1953.

3 Hedgepeth, Joel W. Obtaining Ecological Data in the Sea Chapter 4 in "Treatise on Marine Ecology and Paleoecology" Memoir 67. Geol. Soc. Am. 1963.

4 Isaacs, John D. and Columbus, O.D. Oceanographic Instrumentation NCR Div. Phys. Sci. Publ. 309, 233 pp. 1954.

5 Jackson, H.W. A Controlled Depth Volumetric Bottom Sampler. Prog. Fish Cult., April, 1970.

6 Lagler, Karl F. Freshwater Fishery. Biology, Wm. C. Brown Company. Dubuque. 1966.

7 Larrimore, R.W. Two Shallow Water Bottom Samplers. (in press) 1969.

8 Morgan, A.H. Field Book of Ponds and Streams. G.P. Putnam Sons. New York. 1930.

9 Pennak, R.W. Freshwater Invertebrates of the United States. The Ronald Press Company. New York. 1953.

10 Standard Methods for the Examination of Water and Wastewater. (12th ed. in print; 13th ed. in press, due in print 1970). APHA, AWWA, WPFC. Publ. by Am. Pub. Health Assoc. New York.

11 Sverdrup, H.U. et al. Observations and Collections at Sea. Chapter X in: The Oceans, Their Physics, Chemistry, and Biology. Prentice-Hall, Inc., New York. 1087 pp. 1942.

12 Usinger, R.L. Aquatic Insects of California (Section on Field Methods). University of California Press. Berkeley. 1956.

13 Weber, C.I. The Preservation of Phytoplankton Grab Samples. Trans. Am. Mic. Soc. 87:70. 1968.

14 Welch, Paul S. Limnological Methods. The Blakiston Company, Philadelphia, Pennsylvania. 1948.

15 FWPCA, Investigating Fish Mortalities. USDI, No. CWT-5, 1970. U.S. Gov't. Print. Off. 1970 0-380-257.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

THE SYSTEM OF BIOLOGICAL CLASSIFICATION

I INTRODUCTION

There are few major groups of organisms that are either exclusively terrestrial or generally aquatic. The following remarks apply to both, however, primary attention will be directed to aquatic types.

II CLASSIFICATION

One of the first questions usually posed about an organism seen for the first time is: "what is it?" usually meaning, "what is its name?" The naming or classification of biological organisms is a science in itself (taxonomy). Some of the principles involved need to be understood by anyone working with organisms however.

A Names are the "key number", "code designation", or "file references" which we must have to find information about an unknown organism.

B Why are they so long and why must they be in Latin and Greek? File references in large systems have to be long in order to designate the many divisions and subdivisions. There are over a million and a half items (or species) included in the system of biological nomenclature (very few libraries have as many as a million books to classify).

C Common names are rarely available for most invertebrates and algae. Exceptions to this are common among the molluscs, many of which have common names which are fairly standard for the same species throughout its range. This may be due to their status as a commercial harvest or to the activities of devoted groups of amateur collectors. Certain scientific societies have also assigned "official" common names to particular species; for example, aquatic weeds - American Weed Society; fish - American Fisheries Society; amphibians (salamanders and frogs) - American Society of Ichthyologists and Herpetologists.

D The system of biological nomenclature is regulated by international congresses.

1 It is based on a system of groups and super groups, of which the foundation (which actually exists in nature) is the species.

2 The taxa (categories) employed are as follows:

The species is the foundation (plural: species)

Similar species are grouped into genera (singular: genus).

Similar genera are grouped into families.

Similar families are grouped into orders.

Similar orders are grouped into classes.

Similar classes are grouped into phyla (phylum).

Similar phyla are grouped into kingdoms.

Other categories such as sub-species, variety, strain, division, tribe, etc. are employed in special circumstances.

D The scientific name of an organism is its generic name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names).

1 The generic (genus) name is always capitalized and the species name written with a small letter. They

should also be underlined or printed in italics when used in a technical sense. For example:

Homo sapiens - (=sentiens) modern man

Homo heidelbergensis - heidelberg man

Homo neanderthalis - neanderthal man

Oncorhynchus gorbuscha - pink salmon

Oncorhynchus kisutch - coho salmon

Oncorhynchus tshawytscha - chinook salmon

2 Common names do not exist for most of the smaller and less familiar organisms. For example, if we wish to refer to members of the genus Gomphonema (a diatom) we must simply use the generic name, and:

Gomphonema olivaceum

Gomphonema parvulum

Gomphonema abbreviatum

three distinct species which have different significances to algologists interpreting water quality.

3 A complete list of the various categories to which an organism belongs is known as its "classification". For example, the classification of a type of diatom and a midge larva or "bloodworm" are shown side by side below. Their scientific names are Gomphonema olivaceum and Chironomus riparius.

a Examples of the Classification of an animal and a plant:

Kingdom	Plantae	Animalia
Phylum	Chrysophyta	Arthropoda
Class	Bacillariophyceae	Insecta
Order	Pennales	Diptera
Family	Gomphonemaceae	Chironomida
Genus	Gomphonema	Chironomus
Species	olivaceum	riparius

b These seven basic levels of organization are often not enough for the complete designation of one species among thousands; however, and so additional echelons of terms are provided by grouping the various categories into "super..." groups and subdividing them into "sub..." groups as:

Superorder, Order, Suborder, etc. Still other category names such as "tribe", "division","variety", "race", "section", etc., are used on occasion.

c Additional accuracy is gained by citing the name of the authority who first described a species (and the date) immediately following the species name. Authors are also often cited for genera or other groups.

d A more complete classification of the above midge, follows:

Kingdom	Animalia	
Superphylum	Annelid	
Phylum	Arthropoda	
Class	Insecta	
Order	Diptera	
Suborder	Nematocera	
Family	Chironomidae	
Subfamily	Chironominae	
Tribe	Chironomini	
Genus	Chironomus	
Species	riparius Meigen	1804

e It should be emphasized that since all categories above the species level are essentially human concepts, there is often divergence of opinion in regard to how certain organisms should be grouped. Changes result as knowledge grows.

f The most appropriate or correct names too are subject to change. The species itself, however, as an entity in nature, is relatively timeless and so does not change to man's eye.

This outline was prepared by H. W. Jackson, Chief Biologist and R. M. Sinclair, Aquatic Biologist, National Training Center, Office of Water Programs, Cincinnati, OH 45268.

TECHNIQUES OF PLANKTON ANALYSIS

I INTRODUCTION

A A planned program of plankton analysis should involve periodic sampling at weekly intervals or more often.

1 Most interference organisms are small, and hence have relatively short life histories.

2 Populations of such organisms may fluctuate rapidly in response to changing water, weather, or seasons.

3 Seasonal growth patterns of plankton tend to repeat themselves from year to year, thus they are relatively predictable.

B A well planned study or analysis of the growth pattern of plankton in one year will provide a basis for predicting conditions the following year.

1 Since the seasons and the years differ, the more records are accumulated, the more useful can they become.

2 As the time for an anticipated bloom of some trouble maker approaches, the frequency of analyses may be increased.

C Detection of a bloom in its early stages will facilitate more economical control.

II FIELD ASPECTS OF THE ANALYSIS PROGRAM

A Two general aspects of sampling are commonly recognized: quantitative and qualitative.

1 Qualitative examination tells what is present.

2 Quantitative tells how much.

3 Either approach is useful, a combination is best.

B Equipment of collecting samples in the field is varied.

1 A half liter bottle will suffice for surface samples if carefully taken.

2 A Kemmerer type sampler is suggested for depth samples.

3 Plankton nets concentrate the sample in the act of collecting, and capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative, however.

4 Other methods such as the Clark-Bumpus sampler or the Juday plankton trap may be employed for special purposes.

C The location of sampling points is important.

1 Both shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 6 inches to one foot.

b "Deep" samples should be taken at depths of 5 to 20 feet, as the depth of the reservoir should have at least one open water sampling point.

c Each major bay or shoal area should have at least one sampling point.

d Additional sampling stations should be established on the basis of experience and resources.

e These samples too may be composited if desired to give an overall summary.

of conditions. Such summaries should be interpreted with care.

f Continuous sampling, or the composition of periodic samples over a 24 hour period is also very useful.

III FIELD PRESERVATION OF SAMPLES

Provisions should be made for the field stabilization of the sample until the laboratory examination can be made.

A Refrigeration or icing is very helpful.

B Preservation by 3-5% formalin is most widely used, but somewhat shrinks animals and makes all forms brittle.

C 70% ethanol is a good preservative but extracts the chlorophyll from plants, and hence is not generally recommended.

D Lugols solution is used by some.

E Ultra-violet sterilization is sometimes used in the laboratory to retard the decomposition of plankton.

F A dilute thimerosal preservative solution is currently being used by the National Water Quality Network. It is satisfactory for preservation up to 6 weeks in winter, 1 month in summer.

IV RECEPTION AND PREPARATION OF SAMPLES

A Preliminary sampling and analysis is an essential preliminary to the establishment of a permanent or semi-permanent program.

B Concentration or sedimentation of preserved samples may precede analysis.

1 Batch centrifuge

2 Continuous centrifuge

3 Sedimentation of nannoplankton

C Unpreserved (living) samples should be analyzed at once or refrigerated for future analysis.

D Samples preserved with thimerosal should not be held for more than a month in summer weather, or six weeks in winter.

V SAMPLE ANALYSIS

A Microscopic examination is most frequently employed in the laboratory to determine what plankton organisms are present and how many there are:

1 Optical equipment need not be elaborate but should include:

a Compound microscope with the following equipment:

1) Mechanical stage
2) Ocular: 10X, with Whipple type counting eyepiece (reticule)
3) Objectives:
approx. 10X(16 mm)
approx. 20X(8 mm)
approx. 40X(4 mm)
approx. 95X(1.8 mm)
(Binocular eyepieces are optional)
4) Stage micrometer

b Inverted microscopes offer certain advantages but are not widely available. The same is true of some of the newer optical systems such as phase contrast microscopy. These are often excellent for specialized research work, but are not currently to be recommended for routine water plant use.

2 Precision made counting chambers are required for quantitative work with liquid mounts.

3 Previous to starting serious analytical work, the microscope should be calibrated.

4 Automatic particle counters may be useful for coccoid organisms, but have not yet been satisfactory for general plankton counting.

B Quantitative Plankton Counts

1 All quantitative counting techniques involve the filling of a standard cell of known dimensions with either straight sample or a concentrate thereof.

2 The organisms in a predetermined number of microscope fields or other known area are then observed, and by means of a suitable series of multiplier factors, projected to a number or quantity per ml. gallon, etc.

3 Direct counting of the unconcentrated sample eliminates manipulation, saves time, and reduces error. If frequency of organisms is low, more area may need to be examined or concentration of the sample may be in order.

4 More conventional techniques employing concentration of the sample provide more organisms for observation, but because they involve more manipulations, introduce additional errors and take more time.

C Several methods of counting plankton are in general use.

1 The numerical or clump count is regarded as the simplest.

2 The areal standard unit method is becoming increasingly popular with waterworks operators.

3 The cubic standard unit method is a logical extension of the areal method, but has not achieved wide acceptance.

D Qualitative "counts" are essentially lists of the kinds of organisms found.

E Relative or proportional counts of special groups are often very useful. For example, diatoms.

F Plankton are sometimes measured by means other than microscopic counts.

1 Settled volume of killed plankton in an Imhoff cone may be observed after a standard length of time. This will evaluate only larger forms and is not to be generally recommended.

2 A gravimetric method employs drying at 60°C for 24 hours followed by ashing at 600°C for 30 minutes. This is particularly useful for chemical and radiochemical analysis.

3 A method for chlorophyll extraction involves filtration, drying for 24 hours, extraction with methyl alcohol, and evaluation in a colorimeter or acetone extracts, using Beckman. This is widely used in "productivity" studies.

4 The membrane (molecular) filter is coming to be widely used. Membranes can be cleared with immersion oil and organisms deposited thereon observed directly after 24 hours, or collected material can be washed off and observed immediately.

VI RECORDS

A Field conditions greatly affect the plankton, and a record thereof should be carefully identified with the collection.

1 Time of day, turbidity of the water, and relative cloudiness affect the amount of light, which affects the vertical distribution of many forms.

2 Water temperature affects growth rate and behavior.

3 Wind causes water drift, and wave action breaks up colonial forms and disperses accumulations.

4 Other conditions may be usefully recorded if circumstances permit.

VII SUMMARY AND CONCLUSIONS

A The field sampling program should be carefully planned to evaluate all significant locations in the reservoir or stream, giving due consideration to the capacity of the laboratory.

B Records and notes should be made of field conditions and carefully associated with the corresponding laboratory analyses in a permanent file.

C Optical equipment in the laboratory should be calibrated.

D Once a procedure for processing plankton is adopted, it should be used exclusively by all workers at the plant.

E Such a procedure should enable the water plant operator to prevent plankton troubles or at least to anticipate them and have corrective materials or equipment stockpiled.

REFERENCES

1 Clark, W.J., and Sigler, W.F. Method for Concentrating Phytoplankton Samples Using Membrane Filters. J. Limnol. & Oceanog. 8(1): 127-129.

2 Gardiner, A.C. Measurement of Phytoplankton Population by the Pigment Extraction Method. J. Marine Biol. Assoc. 25(4): 739-744. 1943.

3 Hutcheson, George, E. A Treatise on Limnology. John Wiley and Co. New York. 1957.

4 Ingram, W. M., and Palmer, C. M. Simplified Procedures for Collecting, Examining, and Recording Plankton in Water. J. AWWA. 44(7):617-624. 1952.

5 Lackey, J. B. The Manipulation and Counting of River Plankton and Changes in Some Organisms Due to Formalin Preservation. Public Health Reports. 53:2080-93. 1938.

6 Lund, J.W.G., and Talling, J.F. Botanical Limnological Methods with Special References to the Algae. Botanical Review. 23(8&9): 489-583. October, 1957.

7 McNabb, C.D. Enumeration of Freshwater Phytoplankton Concentrated on the Membrane Filter. J. Limn. & Oceanog. 5(1):57-61. 1960.

8 Palmer, C.M. Algae in Water Supplies. U.S. Department of Health, Education, and Welfare, Public Health Service. Publication No. 657. Superintendent of Documents. Washington 25, D.C.

9 Welch, P.S. Limnological Methods. The Blakiston Co. Phila., Toronto. 1948.

10 Williams, L.G. Plankton Population Dynamics. National Water Quality Network Supplement 2. U.S. Public Health Service Publ. No. 663. 1962.

11 Wohlschlag, D.D., and Hasler, A.D. Some Quantitative Aspects of Algal Growth in Lake Mendata. Ecology. 32(4): 581-593. 1951.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, OWP, EPA, Cincinnati, OH 45268.

DETERMINATION OF PLANKTON PRODUCTIVITY

I INTRODUCTION

Primary production is the synthesis of organic matter from inorganic raw materials. The energy required for this process may come from light (photosynthesis), or from chemical sources (chemosynthesis). The primary synthesis of organic matter in lakes and streams is carried on by planktonic and benthic algae and bacteria, and aquatic macrophytes.

II PHOTOSYNTHESIS

The photosynthetic process involves the uptake of CO_2 and the release of O_2. The reactions are enzyme catalyzed and are affected by the following factors:

A Temperature

B Light Intensity

C Light Quality

D pH

E Nutrients

F Trace Elements

III MEASURING PRODUCTIVITY

Methods employed to measure plankton productivity are:

A Standing Crop

B Oxygen

C pH

D Carbon-14

IV STANDING CROP METHOD

The productivity of a body of water is indicated, in a general way, by the density of the plankton population. The standing crop of plankton is commonly measured by determining one or more of the following:

A Dry and Ash-free Weight of Seston

B Cell or Unit Counts

C Cell Volume

D Chlorophyll

E Particulate and Dissolved Carbohydrate

F Particulate and Dissolved Organic Carbon

Increases in the standing crop over a period of time may be used to determine productivity. However, this method provides only a rough approximation of the rate of primary production.

V OXYGEN METHOD

The use of dissolved oxygen to determine short-term rates of primary production was introduced by Gaardner and Gran (1927). Estimates of the amount of carbon fixed are based on the premise that one molecule of oxygen is given off for each atom of carbon assimilated.

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

A "Light" and "dark" bottles are filled with sample and resuspended at various depths for 4 - 24 hours.

B The concentration of dissolved oxygen is determined (using the Winkler Method) at the beginning and end of the incubation period. The values obtained are as follows:

1 Final "light" bottle O_2 - initial O_2 = net photosynthesis

2 Initial O_2 - Final "dark" bottle O_2 = respiration

3 Net photosynthesis + respiration = gross photosynthesis

This method has some serious disadvantages:

A The bottles provide an artificial substrate for the proliferation of bacteria which use up large amounts of O_2, resulting in erroneously high respiration and low net photosynthesis values.

B The lower limit of sensitivity of the Winkler Method is 0.02 mg O_2/liter. This is a serious handicap when working in oligotropic lakes and the open sea.

VI CARBON-14 METHOD

The use of carbon-14 for the measurement of the rate of carbon assimilation by phytoplankton was pioneered by E. Steemann Nielsen (1952). The method is simple and very sensitive.

A Carbon-14 labelled sodium bicarbonate (4 - 10μc/liter) is added to "light" and "dark" bottles, which are resuspended in the water for 4 - 24 hours.

B An aliquot of the sample is passed through a membrane filter (1.2 μ pore diameter), and the filters are treated with acid to remove any inorganic labelled carbon.

C The (beta) activity of the filter is determined with an end-window Geiger tube, or with gas flow or liquid scintillation techniques.

D The carbon fixed is determined as follows:

$$\text{carbon fixed} = \frac{\text{activity on filter}}{\text{total activity added}} \times \text{available } HCO_3^- \times \text{correction for isotope discrimination}$$

There are several important disadvantages in this method.

A Some of the labelled photosynthesis products will be broken down immediately by respiration, and the liberated carbon-14 reused in photosynthesis. Therefore, it is generally agreed that the method measures only net photosynthesis.

B It has been found that the algae rapidly excrete up to 50% of the photosynthate in the form of organic acids, carbohydrates, and amino acids. Since these labelled materials are not retained by the filter, they escape detection.

VII pH METHOD

The uptake of CO_2 by the algae during photosyntheses results in an increase in the pH of the surrounding medium. Periodic pH measurements are made of the body of water being studied, and the carbon uptake is determined using published nomographs.

Verduin (1952) used this method in a study of the productivity of Lake Erie. However, the method has not gained wide acceptance because it can be used only in waters with low alkalinity.

REFERENCES

1 Allen, M.B. Excretion of Organic Compounds by Chlamydomonas. Arch. f. Mikrobiol. 24:163-168. 1956.

2 Curl, H. Jr., and Small, L.F. Variations in Photosynthetic Assimilation in Natural Marine Photoplankton Communities. Limnol. Oceanogr. 10(Suppl.):R67-R73. 1965.

3 Gaardner, T., and Gran, H.H. Investigations of the Production of the Plankton in the Oslo Fjord. Rapp. et Proc. - Verb., Con. Internat. Explor. Mer. 42:1-48. 1927.

4 Goldman, C.R. Molybdenum as a Factor Limiting Primary Productivity in Castle Lake, California. Science 132: 1016-1017. 1960.

5 Kamen, M.D. Primary Processes in Photosynthesis. Academic Press, New York. 1963.

6 Marshall, S.M., and Orr, A.P. Carbohydrate as a Measure of Photoplankton. J. Mar. Biol. Assoc. U.K. 42:511-519. 1962.

7 Ryther, J.H. Photosynthesis in the Ocean as a Function of Light Intensity. Limnol. Oceanogr. 1:61-70. 1956.

8 Steemann Nielsen, E. The Use of Radioactive Carbon (C-14) for Measuring Organic Production in the Sea. J. Con. Internat. Explor. Mer. 18:117-140. 1952.

9 Strickland, J.D.H. Measuring the Production of Marine Phytoplankton. Bull. Fish. Res. Bd. Can. No. 122: 1-172. 1960.

10 Verduin, J. Photosynthesis and Growth Rates of Two Diatom Communities in Western Lake Erie. Ecology 33(2): 163-168. 1952.

11 Vernon, L.P. Bacterial Phytosynthesis. Ann. Rev. Plant. Physiol. 15:73-100. 1962

12 Wetzel, R.G. A Comparative Study of the Primary Productivity of Higher Aquatic Plants, Periphyton, and Phytoplankton in a Large, Shallow Lake. Internat. Rev. Hydrobiol. 49:1-61. 1964.

13 Yentsch, Charles S. The Measurement of Chloroplastic Pigments- Thirty Years of Progress? pp. 255-270 in Chemical Environment in the Aquatic Habitat. Proc. IBP Symposium. Amsterdam. 1967. (N.V. Noord-Hollandsche Uitgevers Maatschappij. Amsterdam, Netherlands. 8.95)

This outline was prepared by C. I. Weber, Chief, Biological Methods Branch, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

ARTIFICIAL SUBSTRATES

I INTRODUCTION: THE NATURE OF ARTIFICIAL SUBSTRATES

A Artificial substrates are anything deliberately placed in the water for the purpose of providing a place for benthic or attached (sessile, sedentary, etc.) organisms to grow on or in. This is in contrast to "bait" which is used as an attractant.

B Their origins for commercial use, or human food production are rooted in antiquity. Some examples are:

1 Ropes, poles, brush, concrete structures, and other objects thrust into the bottom, or suspended in estuarine waters to catch and grow oysters and mussels (cultural techniques), known virtually around the world.

2 Straw or reed tepees planted in shallow alkaline lakes (in Mexico for example) to catch the eggs of Corixids (Insecta: Order Hemiptera, back-swimmers). Eggs are harvested by drying and brushing them off onto white sheets. Used for human food.

C The fouling of ships bottoms, piling, etc. by barnacles and other marine life is an "artificial substrate in reverse".

D The use of aggregate to support a zoogloeal mass of micro-biota in a trickling filter, thus simulating a riffle area in a surface stream, is a modern concept to harness and make use of "consumer" and "reducer" elements of a community in order to dissipate the energy (oxidize, exhaust the food value) contained in sewage.

II ECOLOGICAL BASIS

A Artificial substrates are based on the "laws of organismal distribution."

1 Any given kind of organisms tends to be present (inhabit) in all available suitable habitats.

2 Any given habitat tends to be inhabited by all suitably adapted kinds of organisms.

B A "substrate" being an object (or group of objects) constitutes a habitat suitable for sessile or attached organisms, and also those that naturally burrow in, crawl over, or otherwise live associated with objects. Natural objects here could mean the bottom, stones, sticks (floating or sunk), etc.

C Organisms that would not be attracted to substrates would be plankton and nekton (fish and larger swimming invertebrates).

D Ecological Succession

Colonization is rapid in a biologically productive water, and normally reaches a stable climax* community in about a month. A typical outline of successive forms to appear in a freshwater situation, for example, might be as follows.

1 Periphyton (slime forming) stage (see also below)

a Bacteria - within an hour

b Diatoms - within the first day

c Other micro-algae - within the first day

d Protozoa - within the first day

2 Macroinvertebrate dominated stage (see also below)

a Primary attached or sedentary colonizers - second to third day

1) Net caddisflies

2) Bryozoa

3) *Cordylophora caspia*

4) *Hydra*

---

*A community which has achieved a point of no further change, under a given set of environmental conditions. Time scale may vary with circumstances.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

b Primary foragers

1) Mayflies

2) Stoneflies

3) Midges

c Secondary attached or sedentary colonizers:

1) Sponges

2) Filamentous algae

d Adventitious forms

1) Crustaceans

2) Flatworms

3) Leeches

4) Snails

5) Other

3 Artificial substrates in a marine environment proceed through similar stages, except that the macroinvertebrate stage may be more subject to variation in the attachment of broods of barnacle, oyster, and other larvae resulting from greater numbers of types present, tidal current variation, meteorological conditions, etc.

III ARTIFICIAL SUBSTRATES AS SCIENTIFIC COLLECTING DEVICES

A A review of the history of artificial substrates for collecting microorganisms (aufwuchs) (Cooke, 1956) indicates that glass microscope slides were first used for this purpose about 1915. Wood or metal panels appear, however, to have been deliberately exposed for the scientific collection of larger organisms at least since approximately the turn of the century, and probably long before that (Visscher, 1928).

B Biological Applications

The principles of the artificial substrate remain the same, regardless of the community sampled. Two general types of communities and associated samplers have been employed:

1 Periphyton (or aufwuchs) samplers

Periphyton is the community of slime forming microorganisms which is the first to attach to objects newly exposed under water. This community is generally considered to provide an anchor layer to which other higher forms of life can more readily attach. It tends to persist until overgrown or displaced by larger organisms, and then in turn can be found spreading over the surfaces of these same larger plants and animals.

2 Periphyton has been widely studied as it appears on 1 × 3 glass microscope slides which are equally convenient to expose in the field and to study in the laboratory.

3 Particular studies have included:

a The original bacterial and fungal slime

b Diatom identification and counts

c Identification and counts of other microscopic algae

d Protozoans

e Primary productivity

4 The macroinvertebrate community is sampled by a great variety of devices such as those cited below. The organisms are usually removed from the substrate for study. Applications have included the following:

a General study of the macroinvertebrate community b Estimates of productivity

c Studies of the life cycle of particular species

d Studies of the influence of the substrate on the attachment of sessile forms

1) The influence of toxic paints for the prevention of fouling organisms

2) Wood panels to study the penetration of boring molluscs and crustaceans

C Effect of type of device on what is collected

1 Wood boring organisms like teredo worms (Mollusca, Pelecypoda) or gribbles (Arthropoda, Isopoda) would obviously be attracted primarily to wood (although some are known to bore in other materials).

2 Delicate forms and crawling forms would be most likely to be collected on devices having a shape to protect against strong currents.

3 Those with strong attachments could endure swift currents; often, surprisingly, even during periods of original attachment (ex. byssus attached clams which are also benthic forms).

4 Bottom burrowers would be most likely collected in artificially contained portions of bottom material.

D Effect of Location

1 The depth at which a sampler is suspended may influence the organisms attracted.

2 Location in or out of a current, direct sunlight, etc., will influence the take.

E Some Types of Devices

1 Cement plates, panels, and blocks

2 Ceramic tiles

3 Wood blocks

4 Metal plates

5 Glass slides - 1 × 3 inch micro slides are used by many workers. Numerous devices are employed to hold them. They are generally either floated (Weber and Raschke 1966) or suspended in racks, anchored to submerged bricks or other objects.

6 Plastic petri dishes

Burbanck and Spoon utilized an ordinary 50 × 12 mm plastic petri dish for collecting sessile protozoa. Sickle modified this by using a styrofoam cup (6 oz. size) with the bottom third being cut off. The lower unit of the plastic dish is easily wedged into place in the cup and the device is simply held by a nylon line on a rope held in place by an appropriate anchor and float.

The cup which tends to float is so held that the petri dish bottom is in a horizontal position and bottom side up.

7 Multiple plate (Hester and Dendy, 1962)

a Common current procedure utilizes 3-inch squares of 1/4 inch thick Masonite separated by 1-inch square spacers.

These may be:

b Threaded on an eye bolt or long rod.

c Suspended by a loop of nylon cord.

8 Baskets or trays of bottom-type material

a Trays of bottom material sunk in the surface layer of the bottom.

b Baskets of stones suspended in the water (Anderson and Mason, 1966).

9 Boxes, cages, bundles, etc., of brush, reeds, or artificial material.

10 Polyethylene tapes

11 Plastic webbing

Minnesota Mining and Manufacturing Company conservation web no. 200.

12 Styrofoam

13 Glass coverslips

Small slips are floated on the surface of the water. Highly useful for protozoa and rotifers. Remove and place on a micro slide. Examine as a wet mount.

F Retrieval is an acute problem with all of these samplers.

1 Physical factors

a Relocation

b Floods and drift

c High water

2 Well marked samplers or floats are naturally vulnerable to the public, resulting in disturbed, damaged, or destroyed sample gear.

a This has been overcome by an ingenious submerged float and recovery line device. The weak link in a submerged recovery line is a modified flash bulb. An electronic device actuated by an underwater gun breaks the bulb allowing the float and attached line to surface. (Ziebell, McConnell, and Baldwin)

b This unit has been further modified by Fox (University of Georgia Cooperative Fishery Unit) who used an inexpensive detonator, "Seal Salute". The latter is an inexpensive fused charge designed for underwater explosion.

IV ARTIFICIAL SUBSTRATES OR SAMPLERS, AND WATER QUALITY

A Artificial substrates provide a habitat ("place to live"). It follows from the laws of distribution (II A 1 and 2 above), that the community which inhabits a device will be governed by the physical nature or structure interacting with the characteristics of the surrounding water (velocity, temperature, chemical characteristics, etc.). Since the nature of the sampler is controlled, it is evident that the characteristics of the water constitute the variable factor.

B Water Quality Surveillance

1 Similar substrates suspended side by side in the same water tend to accumulate (essentially) the same communities and quantities of organisms.

2 Similar substrates suspended in different waters accumulate different communities and quantities.

3 Ergo: different communities and quantities collected from similar substrates at different places and times, probably indicate different water qualities.

a These may be natural (seasonal, diurnal, etc.)

b Or they may be a result of human influences (pollution)

c A series of samplers the length of a stream, lake, or estuary can suggest "steady state" differences in water quality.

d A series of samplers exposed over a period of time at a given site can suggest changes of water quality in time.

4 The artificial substrate thus essentially constitutes an in-situ bioassay of the water.

C Interpretation and Significance of Collections

1 The unit of comparison is most appropriately taken as "the sampler". The artificial substrate by definition is not the natural local bottom material, and unless it consists of a portion of that bottom which has been actually removed and replaced in an artificial container (III-D-7) the composition and magnitude of the community it contains may or may not bear a definitive relationship to the actual natural problem. The take of the artificial substrate thus may have relatively little relationship to the take of a Petersen or an Ekman grab (dredge).

2 Comparisons between different types of samplers are likewise hazardous. Each is what it is, and if they are different they are not identical; thus the biota each collects cannot be expected to be identical (CF: II A).

3 Artificial substrates should generally be compared on a "sampler vs sampler" basis, or for periphyton, "unit area vs unit area".

REFERENCES

1 Anderson, J.B. and Mason, William T. Jr. A Comparison of Benthic Macroinvertebrates collected by Dredge and Basket Sampler. Jour. Water Poll. Cont. Fed. 40(2):252-259.

2 Arthur, John W. and Horning, W.B., II. The Use of Artificial Substrates in Pollution Surveys. Amer. Midl. Nat. 82(1):83-89.

3 Besch, W., Hoffman, W., and Ellenberger, W. Das Macrobenthos auf Polyatchylensubstraten in Fliessgswasseren. Annals de Limnologic. 3(2):331-367. 1967.

4 Burbanck, W.D. and Spoon, D.M. The Use of Sessile Ciliates Collected in Plastic Petri Dishes for Rapid Assessment of Water Pollution. J. Protozool. 14(4):739-744. 1967.

5 Cooke, William B. Colonization of Artificial Bare Areas by Microorganisms. Bot. Rev. 22(9):613-638. Nov. 1956.

6 Fox, Alfred C. Personal Communication. 1969.

7 Hester, F.E. and Dendy, J.S. A Multiple-Plate Sampler for Aquatic Macroinvertebrates. Trans.Am. Fish. Soc. 91(4):420-421. April 1962.

8 Hilsenhoff, William L. An Artificial Substrate Device for Sampling Benthic Stream Invertebrates. Limnology and Oceanography. 14(3):465-471. 1969.

9 Mason, W.T., Jr., Anderson, J.B., and Morrison, G.E. A Limestone-Filled, Artificial Substrate Sampler Float Unit for Collecting Macroinvertebrates in Large Streams. Prog. Fish-Cult. 29:74. 1967.

10 Ray, D.L. Marine Boring and Fouling Organisms. University of Washington Press, Seattle. pp 1-536. 1959.

11 Sickel, James B. A Survey of the Mussel Populations (Unionidae) and Protozoa of the Altamaha River with Reference to their Use in Monitoring Environmental Changes. MS Thesis. Emory University. 133 pp. 1969.

12 Sladeckova, A. Limnological Investigation Methods for the Periphyton ("Aufwuchs") Community. Bot. Rev. 28(2):286-350. 1962.

13 Spoon, D.M. and Burbanck, W.D. A New Method for Collecting Sessile Ciliates in Plastic Petri Dishes with Tight Fitting Lids. J. Protozool. 14(4):735-739. 1967.

14 Visscher, J. Paul. Nature and Extent of Fouling of Ships Bottom. Dept. Comm. Bur. Com. Fish. Doc. No. 1031. pp 193-252. 1928.

15 Weber, C.E. and Rauschke, R.L. Use of a Floating Periphyton Sampler for Water Pollution Surveillance. Water Poll. Sur. Sept. Applications and Develop. Report No. 20. FWPCA-USDI, Cincinnati, Ohio. September 1966.

16 Wene, George and Wickliff, E. L. Modification of a Stream Bottom and its Effect on the Insect Fauna. Canadian Entomologist. Bull. 149, 5 pp. 1940.

17 Ziebell, Charles D., McConnell, W. J., and Baldwin, Howard A. A Sonic Recovery Device for Submerged Equipment. Limnol. and Ocean. 13(1):198-200. 1968.

This outline was prepared by H. W. Jackson, Chief Biologist and R. M. Sinclair, Aquatic Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

ARTIFICIAL AND RELATED SUBSTRATES - REFERENCES

INTRODUCTION

This reference list in the field of artificial substrates has been prepared for those not familiar with the literature and for those planning to use one or more of these techniques. It includes references both to periphyton and benthos including techniques, rationale, and practical applications. Since 1967, Dr. C. I. Weber of the Analytical Quality Control Laboratory has compiled an annual bibliography for the Midwest Benthological Society. These bibliographies are recommended for additional and current references. This list is thus complementary to Weber's annual citations for the periphyton. Addresses and sources are given where possible.

Priority has been given to comprehensive works; those of historical note, especially where the original description of a sampler is given; and a sampling of recent papers to show versatility and application for general and specific uses.

GENERAL

Cooke, William B. Colonization of Artificial Bare Areas by Microorganisms. Bot. Rev. 22(9):613-638. Nov. 1956.

Sladeckova, Alena. Limnological Investigation Methods for the Periphyton ("Aufwuchs") Community. Bot. Rev. 28(2):286-350. 1962.

Cummins, K. W. An Evaluation of Some Techniques for the Collection and Analysis of Benthic Samples with Special Emphasis on Lotic Waters. American Midland Naturalist, Vol. 67, No. 2. pp. 477-504. 1962.

PERIPHYTON - GENERAL

Dumont, H.J. A Quantitative Method for the Study of Periphyton. Limnol. Oceanogr. 14(2):303-307. 1969.

Weber, C. E. (EPA, 1014 Broadway Cincinnati, OH 45202) Benthic Macroinvertebrates and Periphyton. Select and Current Bibliographies. Midwest Benthological Society.

Sladeckova, Alena. The Significance of the Periphyton in Reservoirs for Theoretical and Applied Limnology. Verh. Internat. Verein. Limnol. 16:753-758. 1966

PERIPHYTON - PARAFFIN-COATED SUBSTRATES

Beers, G. D. and Neuhold, J. M. Measurement of Stream Periphyton on Paraffin-Coated Substrates. Limnol. and Oceanogr. 13(3):559-562. 1968.

PERIPHYTON - GLASS SLIDES

See general references above.

Thomas, Nelson A. (National Center for Field Investigations, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213.) Method for Slide Attachment in Periphyton Studies. Manuscript. (Nonhardening "Plasti-tak" used for attaching slides to bricks.)

Weber, Cornelius I., and McFarland, Ben H. (EPA, Analytical Quality Control, 1014 Broadway, Cincinnati, OH 45202) Periphyton Biomass-Chlorophyll Ratio as an Index of Water Quality. 17th Annual Meeting, Midwest Benthological Society. 19 pp. Multilithed. 1969.

Weber, C. E. and Rauschke, R. L. Use of a Floating Periphyton Sampler for Water Pollution Surveillance. Water Poll. Sur. Sept. Applications and Develop. Report No. 20. FWPCA-USDI, Cincinnati, Ohio. September 1966.

PERIPHYTON - DIATOMS

Patrick, R., Hohn, M. H., and Wallace, J. H. A New Method for Determining the Pattern of the Diatom Flora. Notulae Naturae, Academy of Natural Sciences of Philadelphia. No. 259. pp. 1-12. 1954.

Hohn, M. H. The Use of Diatom Populations as a Measure of Water Quality in Selected Areas of Galveston and Chocolate Bay, Texas. Publications of the Institute of Marine Science of the University of Texas. Vol. 6. pp. 206-212. 1959.

Patrick, R. Factors Affecting the Distribution of Diatoms. Botanical Review. Vol. 14. No. 8. pp. 473-524. 1948.

Patrick, R. A Discussion of Natural and Abnormal Diatom Communities. In: Jackson, D. F. (ed.), Algae and Man. Plenum Press, New York. pp. 185-204. 1964.

Hohn, M. Determining the Pattern of the Diatom Flora. Journal of the Water Pollution Control Federation, Vol. 33. No. 1. pp. 48-53. 1961.

Hohn, M. The Relationship Between Species Diversity and Population Density in Diatom Populations from Silver Springs, Florida. Transactions of the American Microscopical Society. Vol. 80. No. 2. pp. 140-165. 1961.

PERIPHYTON - PLASTIC SLIDES AND PANELS

Grzenda, Alfred R. and Brehmer, Morris L. A Quantitative Method for the Collection and Measurement of Stream Periphyton. Limnol. Oceanog. 5(2):190-194.

PERIPHYTON - DIATOMS - STYROFOAM

Hellerman, J. A Study of the Diatoms of Mohonk Lake, New York and Vicinity. Unpublished M.S. Thesis. Rutgers - The State University. 1962.

Hohn, Matthew H. (Department of Biology, Central Michigan University, Mt. Pleasant, Michigan). Artificial Substrate for Benthic Diatoms--Collection, Analysis, and Interpretation. p.87-97. 1966. In: Organism Substrate Relationships in Streams Pymatuning Special Publication. No. 4. $2.50. Pymatuning Laboratory of Ecology, University of Pittsburgh. Pittsburgh, PA 15213.

PERIPHYTON - TRICKLING FILTERS

Cooke, W. B. Continuous Sampling of Trickling Filter Populations. Sewage and Industrial Wastes. Vol. 30. No. 1. pp. 21-27. 1958.

Cooke, W. B. Fungi in Polluted Water and Sewage. IV. The Occurrence of Fungi in a Trickling Filter-type Sewage Treatment Plant. Proceedings of the 13th Industrial Waste Conference, Purdue University, Series No. 96, Vol. 43. No. 3. pp. 26-45. 1959.

Cooke, W. B. Trickling Filter Ecology. Ecology. Vol. 40. No. 2. pp. 273-291. 1959.

PERIPHYTON - LABORATORY STUDIES

Ehrlich, Gary G. and Slack, Keith V. (Water Res. Div., USGS, Menlo Park, California 94025). Uptake and Assimilation of Nitrogen Microecological Systems. Spec. Tech. Pub. 448. ASTM. p. 11-23. 1969.

MACROINVERTEBRATES - GENERAL

Macan, T. T. Methods of Sampling the Bottom Fauna of Stony Streams. Mitt. Intern. Ver. Limnol. No. 8. p. 1-21. 1958.

MACROINVERTEBRATES - BRUSH BOXES

Scott, D. C. Biological Balance in Streams. Sewage and Industrial Wastes. Vol. 30. No. 9. pp. 1169-1172. 1958

MACROINVERTEBRATES - ROCK BASKETS

Anderson, J. B. and Mason, William T., Jr. A Comparison of Benthic Macroinvertebrates Collected by Dredge and Basket Sampler. Jour. Water Poll. Cont. Fed. 40(2):252-259.

Anderson, J. B. and Mason, W. T., Jr. The Use of Limestone-Filled Samples for Collecting Macroinvertebrates from Large Streams. Water Poll. Surv. Syst. Application and Develop. Report No. 17, FWPCA-USDI, Cincinnati, Ohio. May 1966.

Henson, E. B., Jr. A Cage Sampler for Collecting Aquatic Fauna. Turtox News. 43:298-299. 1965.

Hilsenhoff, William L. An Artificial Substrate Device for Sampling Benthic Stream Invertebrates. Limnology and Oceanography. 14(3):465-471. 1969.

Mason, W. T., Anderson, J. B., and Morrison, G. E. , Analytical Quality Control, 1014 Broadway, Cincinnati, Ohio) Limestone-filled, Artificial Substrate Sampler-float unit for Collecting Macroinvertebrates in large streams. Progressive Fish Culturist. 29:74. 1967.

Wene, G., and Wickliff, E. L. Modification of the Stream Bottom and Its Effect on the Insect Fauna. Can. Entomologist. 72:131-135. 1940.

MACROINVERTEBRATES - MULTIPLE PLATES

Arthur, John W. and Horning, William B., II. (Federal Water Pollution Control Administration, Duluth, Minnesota, National Water Quality Laboratory. The Use of Artificial Substrates in Pollution Surveys. The Amer. Midland Natur. Vol. 82. No. 1. pp. 83-89. 1969.

Hester, F. E. and Dendy, J. S. A Multiple-Plate Sampler for Aquatic Macroinvertebrates. Trans. Am. Fish. Soc. 91 (4):420-421. April 1962.

Brewer, Jesse W. and Gleason, Gale R. Modification of the Dendy Principle for Stream Bottom Sampling. Midwest Benthological Society. 12th Annual Mtg. Mimeo. 1964.

Dendy, J. S. Living Food for Aquatic Animals. Turtox News 41(10):258-259. 1963.

MACROINVERTEBRATES - CEMENT PLATES

Britt, N. W. New Methods for Collecting Bottom Fauna from Shoals or Rubble Bottoms of Lakes and Streams. Ecology. 36:524-525. 1955.

MACROINVERTEBRATES - BURIED TRAYS AND POT SAMPLERS

Coleman, Mary J. and Hynes, H.B.N. The Vertical Distribution of the Invertebrate Fauna in the Bed of a Stream. Limnol. and Ocean. 15(1):31-40. 1970.

Moon, H. P. Methods and Apparatus Suitable for an Investigation of the Littoral Region of Oligotrophic Lakes. Int. Rev. Hydrobiol. 32:319-333. 1935.

Radford, D. S. and Hartland-Rowe, R. Subsurface and Surface Sampling of Benthic Invertebrates in Two Streams. Limnol. and Oceanogr. 16(1):114-120. 1971.

MACROINVERTEBRATES - PLASTIC TAPES FOR BLACKFLIES

Williams, T. R. and Obeng, L. A Comparison of Two Methods of Estimating Changes in Simulium Larvae Populations, with a Description of a New Method. Ann. Trop. Med. Parasit. 56:358-361. 1962.

MACROINVERTEBRATES - ARTIFICIAL WEEDS

Freshwater Biological Association (The Ferry House. Far Sawrey, Ambleside. Westmoreland, England). 1969.

Thirty-seventh Annual Report. p. 36. Artificial weeds made of strands of polyethylene and polypropylene twine. "Artificial Littorella is more heavily colonized "than the living plant" (invertebrates).

MACROINVERTEBRATES - BRICKS

Elvins, B. J. Investigation of the Animal Population in Polluted Streams. Journ. Inst. Sewage Purif. Part 6. p. 569.

MACROINVERTEBRATES - 10 or 20 Stone Surveys

Chutter, F. M. On the Ecology of the Fauna of Stones in the Current in a South African River Supporting a Very Large Simulium (Diptera) Population. J. App. Ecol. 5:531-561. 1968.

Williams. T. R. and Obeng, L. A Comparison of Two Methods of Estimating Changes in Simulium Larval Populations, with a Description of a New Method. Ann. Trop. Med. Parasit. 56:358-361. 1962.

Reed, Roger J. 1966. Some Effects of DDT on the Ecology of Salmon Streams in Southeastern Alaska. USFWS Special Scientific Report No. 542. 15 pp.

Cope, Oliver B. Effects of DDT Spraying for Spruce Budworm on Fish in the Yellowstone River System. Trans. Amer. Fish. Soc. 90:239-251.

MACROINVERTEBRATES - ECOLOGY

Waters, T. F. Recolonization of Denuded Stream Bottom Areas by Drift. Transactions of the American Fisheries Society, Vol. 93, No. 3, pp. 311-315. 1964.

Waters, T. F. Standing Crop and Drift of Stream Bottom Organisms. Ecology. 42:532-537. 1961.

Driscoll, E. G. Attached Epifauna-Substrate Relations. Limnol. Oceanogr. 12(4):633-641. 1967.

MICROINVERTEBRATES - PROTOZOA

Cairns, John, Jr. and Ruthven, Jeanne A. Artificial Microhabitat Size and the Number of Colonizing Protozoan Species. Trans. Amer. Micros. Soc. 89:100-109 (Polyurethane Blocks) 1970.

Spoon, D. M. and Burbanck, W. D. A New Method for Collecting Sessile Ciliates in Plastic Petri Dishes with Tight Fitting Lids. J. Protozool. 14(4):735-739. 1967.

Burbanck, W. D. (Emory University, Atlanta, Georgia) and Spoon, D. M. The Use of Sessile Ciliates Collected in Plastic Petri Dishes for Rapid Assessment of Water Pollution. J. Protozool. 14(4):739-744. 1967.

Sickel, James B. A Survey of the Mussel Populations (Unionidae) and Protozoa of the Altamaha River with Reference to Their Use in Monitoring Environmental Changes. MS Thesis. Emory University. 133 pp. 1969.

Sladecova, Alena. (Inst. Chem. Tech., Prague, Czechoslovakia) Factors Affecting the Occurrence and Stratification of Sessile Protozoans in Artificial Reservoirs. (in Russian,Summ. in English and Czech) Technology of Water. 8(1):483-490. 1964.

SUBSTRATES - BUOYS AND OTHER NAVIGATIONAL AIDS

Miller, Milton A. Isopoda and Tanaidacea from Buoys in Coastal Waters of the Continental U. S., Hawaii, and the Bahamas (Crustacea). Proc. U. S. Nat. Mus. 125 (3652):53 pp. 1968.

Fremling, C. R. Biology and Possible Control of Nuisance Caddisflies of the Upper Mississippi River. Agricultural and Home Economics Experiment Station, Iowa State University of Science and Technology, Ames, Iowa. Research Bulletin 483, pp. 856-879. 1960.

PERIPHYTON-BIOLOGICAL FLOCS

Meyers, Samuel P. (Dept. of Food Science and Tech. Louisiana State Univ., Baton Rouge, LA 70803). 1968. Observations on the Physiological Ecology of Marine Fungi. Bull. Misaki Marine Biol. Inst. Kyoto Univ. 12:207-225.

Meyers, Samuel P. and Hopper, Bruce E. Studies on Marine Fungal-Nematode Associations and Plant Degradation. Helgolander Wiss. Meeresunters 15:270-281 (Cotton Fiber Filter Tubes) 1967.

Phaup, John D. and Gannon, John. Ecology of *Sphaerotilus* in an Experimental Outdoor Channel. *Water Research.* 1:523-541. 1967 (Nylon Yarn Strings) Pergammon Press.

RECOVERY DEVICES

Ziebell, Charles D., McConnell, W. J. and Baldwin, Howard A. A Sonic Recovery Device for Submerged Equipment. Limnol. and Ocean. 13(1):198-200. 1968.

Fox, Alfred C. (Univ. of Georgia, Cooperative Fisheries Unit, Athens, Georgia) Personal Communication. Use of Inexpensive Detonator; "Seal Salute." Miller Fireworks and Novelty Company, 501 Gleneary Road, Holland, Ohio 43528. $4.00 gross and shipping. 1969.

ARTIFICIAL SUBSTRATES - PRACTICAL APPLICATIONS IN WATER POLLUTION CONTROL

Taylor, Mahlon P. Thermal Effects on the Periphyton Community in the Green River. TVA, Div. of Health and Safety, Water Quality Branch. 1967.

Waters, T. F. Notes on the Chlorophyll Method of Estimating the Photosynthetic Capacity of Stream Periphyton. Limnol. Oceanogr. 6:486-488. 1961.

Wetzel, R. G. Techniques and Problems of Primary Productivity Measurements in Higher Aquatic Plants and Periphyton. In: C. R. Goldman et. "Primary Productivity in Aquatic Environments." Univ. Calif. Press. Berkeley. 1966.

U. S. Dept. of the Interior, FWPCA. Keup, Lowell and Stewart, Keith. National Field Investigations Center, 4676 Columbia Parkway, Cincinnati, OH 45226. Effects of Pollution on Biota of the Pigeon River, North Carolina and Tennessee. 35 pp. 1966. Utilization of glass slides attached to bricks for periphyton, chlorophyll biomass ratios to evaluate paper mill and other industrial effluents.

---- Keup, Lowell. National Field Investigations Center. Effects of Pollution on Aquatic Life Resources of the South Platte River Basin in Colorado. Vol. 1 and Vol. 2. 1967.

Vollenweider, Richard A. et al. (Eds.) A Manual on Methods for Measuring Primary Production in Aquatic Environments. International Biological Programme Handbook. No. 12. 213 pp. Davis. 1969.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

ARTIFICIAL SUBSTRATE RECORD SHEET

Stream/lake/estuary ______________________ **Station No.** ____

Date placed _____ **19**__ **Placed by** ________ **Approx. date to recover** ______

Date recovered _____ **Recovered by** ________ **Analysis by** ____________

Analyses requested ______________ ______________

______________ ______________

______________ ______________

Exposure period desired __________ **Exposure days** ____________

Notes ____

No.	Type Code*	Exact Location (coordinates, bearings, etc.)

sketch location in detail

*** 1 = slides 2 = slides on bricks 3 = plate sampler 4 = basket 5 = other** __________

CODE	TAXA	NO.

CODE	TAXA	NO.

Tolerance Status: 0 = tolerant 1 = mod. tolerant 2 = intolerant
Abundance: A = abundant D = dominant C = common F = few
NOTE: This sheet is intended only as a field and lab training aid in specific FWQA training courses. Modified from Tennessee Department of Public Health, Steam Pollution Control Board, Michigan Water Resources Commission, and Georgia Water Quality Control Board.

Column A Tolerance Status B Taxa C Relative abundance or no/ft^2

PROCEDURES FOR FISH KILL INVESTIGATIONS

I INTRODUCTION

Fish kills in natural waters, though unfortunate, can in many instances indicate poor water quality leading to investigations which may improve water quality. Prompt investigations should be organized and conducted so that the resultant data implicates the correct cause. Fish kills tend to be highly controversial, usually involving the general public as well as a number of agencies. Therefore, the investigator can expect his findings to be disputed, quite possibly in a court of law.

The following procedures are presented as a working guide for investigating and reporting fish kills as developed by the personnel of The Lower Mississippi River Comprehensive Project (FWPCA).

II TYPES AND EXTENT OF FISH KILLS

A Natural Mortalities - Those which are caused through natural phenomena such as; acute temperature change, storms, ice and snow cover, decomposition of natural materials, salinity change, spawning mortalities, parasites, and bacterial or viral epidemics.

B Man caused fish kills - Produced by environmental changes through man's activity, and may be attributed to municipal wastes, industrial wastes, agricultural activities and water control activities.

C One dead fish in a stream may be called a fish kill; however, in a practical sense some minimal range in number of dead fish observed plus additional qualifications should be used in reporting and classifying fish kill investigations. The following definitions should be used as guidelines in reporting fish kill investigations. These qualifications are based on a stream approximating 200 feet in width and 6 feet in depth. For other size streams, adjustments should be made.

1 Minor fish kill considered here as NO fish kill and reported so: 1 - 100 dead or dying fish confined to a small area or stream stretch. Providing this is not a reoccurring or periodic situation. For example, near a waste outfall in which stream dilution plays its part and nullifies the effect of the deleterious material. If this is a reoccurring situation, it could be of major significance and, therefore, investigated.

2 Moderate fish kill: 100 - 1000 dead or dying fish observed. In a stream where dilution has had the chance to play its role involving a mile or so of stream, a number of species are affected; and apparently normal fish can be collected immediately downstream from the observed kill area.

3 Heavy fish kill: 10,000 fish or more observed dead or dying. In a stream where dilution has had the chance to play its part, but ten miles or more of the stream are involved, many species of fish are affected and dying fish may still be observed downstream.

III PREPARATION FOR FIELD INVESTIGATION

A Secure maps of area to be investigated.

1 U.S. Geological Survey maps

a 1/250,000 scale for general location

b 1/24,000 for accurately defining the kill area in the field

2 Navigation maps (appropriate agency)

3 Other sources

4 From the data received from the reporting agency, locate the kill area on the map.

a Determine best access points.

b Locate possible known industries, municipalities, or other potential sources of pollution.

c Estimate the possible area to be traveled or inspected on

1) water

2) land

3) both

B Secure sampling equipment and determine size of investigation team needed.

1 Standard equipment to be taken on all investigations (a standard checklist with space for special equipment will often save embarrassment in the field.)

a Thermometer

b Dissolved oxygen sampler

c D.O. bottles

d Winkler D.O. test kit

e Conductivity test meter

f pH test meter or chemical kit

g Sample bottles

h Pencils and note paper

i Current edition of "Standard Methods for the Examination of Water and Wastewater"

2 If preliminary information is available on the possible cause of the kill, consult the latest edition of "Standard Methods" for specific physical and chemical equipment required for collecting, analyzing, or preserving samples possibly containing the suspected causative agent.

3 Form an investigating party

a If only one man is available to make the investigation, preference for choosing the man should be in this order

1) Specialized professional personnel, such as, engineer, chemist, or biologist who is experienced in investigating fish kills and who is capable of adequately reporting the technical aspects of the investigation.

2) A non-specialized professional engineer, chemist, or biologist who has little or no experience in fish kill investigations, but who is capable of adequately reporting the technical aspects of the investigation.

3) A technician who has considerable field experience in pollution and fish kill investigations and who is capable of reporting some of the technical aspects of the investigation.

4) An office technician or other personnel who has had limited field work in pollution investigations.

b If two or more men are needed for the investigation, the party should include at least one person under category (1) above. Preferably, the team should include:

1) A biologist to make a survey of the biological changes.

2) An engineer to make an evaluation of the physical condition of the fish kill area and to make an investigation of an industry or a municipal wastewater treatment plant if needed.

c If a fish kill is observed in its initial state in the field by any one of the people listed under the classification in Section B.3.a., the project office should be informed immediately (after working hours the project director or deputy director should be informed) so that an adequately equipped, specialized investigating party can be formed if needed.

C Contact personnel of the laboratory or laboratories which will participate in analyzing samples. If possible estimate the following and record on sample form No. 1.

1 The number and size of samples to be submitted

2 The probable number and types of analyses required

3 The dates the samples will be received by the laboratory

4 Method of shipment to the laboratory

5 To whom the laboratory results are to be reported

6 The date the results are needed

IV MAKING THE FIELD INVESTIGATION

A Contact the local lay person or official who first observed the kill and reported it.

1 Obtain any additional information which might be helpful which was not reported previously.

2 If possible, retain the reporting party as a guide or invite him to accompany the investigating team.

B Make a reconnaissance of the kill area.

1 Make a decision as to the extent of the kill and if a legitimate kill really has occurred.

2 If a legitimate kill exists take steps to trace or determine the cause.

a Always perform the following physical or chemical tests, during the initial steps of the investigation:

1) Temperature

2) pH

3) Dissolved oxygen

4) Specific conductance

While none of these factors may be directly involved in the kill these tests are performed simply and rapidly in the field and can be used as a baseline or starting point for isolating the cause (s) of the kill.

b Record other physical observations such as:

1) Appearance of water, i.e., turbidity, high algal blooms, oily, unusual appearance, etc.

2) Stream flow pattern, i.e., high or low flow, stagnant or rapidly moving water, tide moving in or out, etc. If possible obtain reading from stream gage if one is near kill area.

3) Weather conditions prevailing at the time of the investigation and information on weather immediately prior to the kill

3 Make a rough sketch or define the kill area on a map so that sampling points, sewer outfalls, etc. can be accurately located on a drawing to be included in a final report.

4 Take close-up and distance photographs of:

a Dead fish in the stream in the polluted area.

b The stream above the polluted area.

c Wastewater discharges.

Photographs will often show a marked delineation between the wastewater discharge and the natural flow of water. Pictures taken at a relatively high elevation, (a bridge as opposed to a boat or from a low river bank) will show more and be more effective. Color photographs are also more effective in showing physical conditions of a stream in comparison to black and white prints.

C Sampling Procedures - The extent and method of sampling will depend upon location and upon the suspected cause of the kill.

1 Stream and wastewater sampling.

a Sample the following points when the pollutional discharge is coming from a well defined outfall.

1) The effluent discharge outfall

2) The stream at the closest point above the outfall which is not influenced by the waste discharge

3) The stream immediately below the outfall

4) Other points downstream needed to trace the extent of the pollution

b The sampling should be extensive enough that when all the data is compiled no question will exist as to the source of the pollution which killed the fish.

c The number of samples to be collected at a given cross section will depend principally on the size of the stream.

1) Streams less than 200 feet wide, not in an industrial area usually can be adequately sampled at one point in a section (Figure 1).

2) Streams 200 feet or wider generally should be sampled two or more places in a section immediately above and below the pollutional discharge; where the pollutional waste has adequately mixed with the stream flow one sample may suffice.

3) A number of samples in a cross section may be required on any size of stream to show that the suspected pollutional discharge is coming from a source located in an industrial or municipal complex (Figure 2).

4) Extensive cross sectional sampling on rivers greater than 200 feet wide will be required for kills involving suspected agricultural or other types of mass runoff.

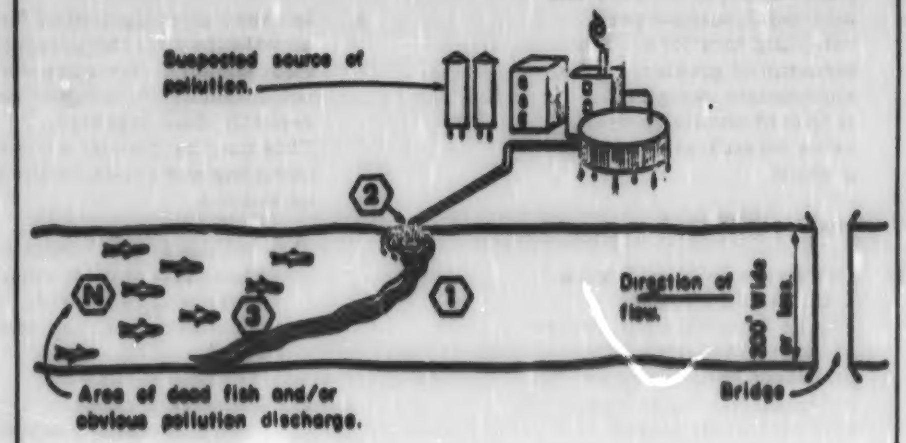

Suspected source of pollution.
2
N
3
1
Direction of flow.
200' wide or less.
Bridge
Area of dead fish and/or obvious pollution discharge.

Figure 1 — Minimum Water Sampling Point On Stream 200 Feet Or Less Wide Involving An Isolated Discharge.

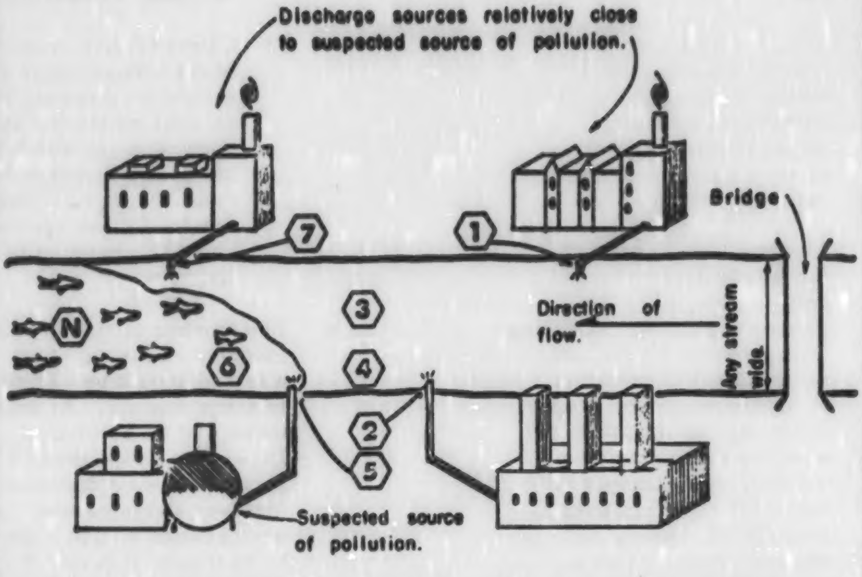

Discharge sources relatively close to suspected source of pollution.
Bridge
7
1
3
N
Direction of flow.
Any stream wide.
6
4
2
5
Suspected source of pollution.

Figure 2 — Minimum Water Sampling Points On A Stream Running Through An Industrial Or Municipal Complex.

PLATE I - RELATIONSHIP OF FISH KILLS TO SOURCE OF TOXICITY

5) Sample depths - On streams 5 feet in depth or less, one mid-depth sample per sampling locations. For streams of greater depths, appropriate sampling judgment should be used, since stratification may be present.

Explanation of Plate I

1) Collection point 1, Figure 1 and points 3 and 4, Figure 2 should be collected as near to the point of pollutional discharge as possible. These points will vary according to stream flow conditions; the pollutional discharges into a slow sluggish stream usually will have a cone of influence upstream of the outfall; whereas, a swift flowing stream usually will not.

2) Collecting an upstream control sample from a bridge within sight of the pollutional discharge would probably be satisfactory in Figure 1 but definitely not in Figure 2.

3) Figures 1 and 2 are given for illustrative purposes only and should be used only as a guide for sampling. Thought must be given to each individual situation to insure adequate, proper sampling. While too many samples are better than too few, effort should be made not to unduly overload the laboratory with samples collected as a result of poor sampling procedures.

2 Biological sampling

a In every investigation of fish or wildlife kills the paramount item should be the immediate collection of the dying or only recently dead organism. This may be done by anyone, sampling and preservation is as follows:

1) Collect 20 plus drops of blood in a solvent rinsed vial, seal same with aluminum foil, cap and freeze.

2) Place bled carcass, or entire carcass if beyond bleeding stage, in plastic bag and freeze. In case no method of freezing is available, icing for a shor period prior to freezing may be acceptable. Labeling of both blood and carcass is important.

3) Controls - live specimens of the affected organisms should be obtained from an area within the same body of water which had not been influenced by the causative agent. Once obtained these specimens should be handled in a like manner.

b The number of individuals involved and the species affected should be enumerated in some manner. At most these will be estimates. Depending on the given situation such as area or distance involved and personnel available enumeration of fish kills may be approached in one of the following ways.

1) For large rivers, establish observers at a station or stations (e.g., bridges) and count the dead and/or dying fish for a specified period of time, then project same to total time involved.

2) For large rivers and lakes, traverse a measured distance of shoreline, count the number and kinds of dead or dying fish. Project same relative to total distance of kill.

3) For lakes and large ponds, count the number and species within measured areas, and then project to total area involved.

4) For smaller streams one may walk the entire stretch involved and count observed number of dead individuals by species.

3 Biological sampling Macro-Invertebrates:

a Sampling of benthic organisms after the more urgent aspects of the kill investigation has been completed can prove to be rewarding relative to extent and cause of kill. Since this general form of aquatic life is somewhat sedentary by nature any release of deleterious materials to their environment will take its toll. Thus by making a series of collections up and downstream, the affected stretch of stream may be delineated when the benthic populations are compared to those from the control area. Also the causative agent may be realized when the specifics of the benthic population present are analyzed.

b Other aspects of the biota which should be considered are the aquatic plants. In lakes and ponds floating and rooted plants should be enumerated and identified. The collection of plankton (rivers and lakes) should be taken in order to determine the degree of bloom, which in itself may cause fish kills because of diurnal DO levels.

c Both aquatic plants and macro-invertebrates may be preserved in a 5% formalin solution.

4 Bioassay

Static bioassay techniques as outlined in Standard Methods may be effectively used to determine acute toxicity of wastes as well as receiving waters.

a In situ using live boxes

b Mobile bioassay laboratory

c Samples returned to Central Lab for toxicity tests

V DETAILED EXAMINATION OF SOURCE OF POLLUTION

A Seven general categories under which causes of kills can be grouped are:

1 Industrial waste discharges

2 Waste discharges from municipal sewerage systems

3 Water treatment plant discharges

4 Agriculture and related activities

5 Temporary activities

6 Accidental spills of oil and other hazardous substances

7 Natural causes

B Industrial Waste Discharges

1 Upon locating the outfall source, collect a sample immediately if possible at the point where the wastes leave the company property.

2 Make an in-plant inspection if possible.

a Contact the plant manager or person in charge.

b Request a brief tour of the facilities.

c Obtain general information concerning the products manufactured; raw materials; manufacturing process; quantities, sources, and characteristics of wastes; and waste treatment facilities if any. Possibly the company may be able to supply a flow diagram or brochure of the plant operations.

d Request specific information concerning the plant operation immediately prior to the start of the kill.

C Waste discharges from a municipal or domestic type sewerage system

1 Discharges from this source may be domestic sewage and industrial wastes combined with domestic sewage. These wastes may be subjected to treatment of a municipal treatment plant or may be discharged directly, untreated to a stream.

2 Generally, the municipality or owner of the sewerage system is held responsible for any discharge in such a system; consequently, after collecting samples, the owner or a representative of the owner of the sewerage system should be contacted. This may be a sewage treatment plant operator, city engineer, public works supervisor, a subdivision developer, etc.

a Obtain information about the operation of the system.

b If the cause of the kill was the result of an industrial waste discharge to a municipal sewer and thence to a stream, information should be obtained from a municipal official about the industry and the problem. An inspection of the industrial plant may be desirable. Generally, this should be done only in cooperation with a municipal official.

D Agriculture and Related Activities

1 Pollution capable of causing fish kills may result from such agricultural operations as crop dusting and spraying fertilizer applications, and manure or other organic material discharges to a stream.

2 Generally, kills related to these factors will be associated with high rains and runoff.

3 The source or type of pollution may be difficult or impossible to locate exactly. It may involve a large area. Talking to local residents may help pinpoint the specific problem area. Runoff from fields, drainage ditches, and small streams leading to the kill area are possible sampling places which may be used to trace the cause.

E Temporary Activities

1 Causes of kills may result from such temporary or intermittent activities as mosquito spraying, construction activities involving chemicals, oils, or other toxic

substances, and weed spraying with herbicide containing materials toxic to fish such as arsenic.

2 As with agricultural activities, tracing the cause of these kills is difficult and may require extensive sampling.

3 Accidental spills from ruptured tank cars, pipelines, etc., and dike collapse of industrial ponds are frequently sources of fish kills.

F Possible Natural Causes of Fish Kills

1 Types of natural causes

a Oxygen depletion due to ice and snow cover on surface waters

b Oxygen depletion at night because of plant respiration or at anytime during the day because of natural occurring organics in the water

c Abrupt temperature changes

d Epidemic and endemic diseases, parasites, and other natural occurring biological causes

e Lake water inversion during vernal or autumnal turnover which results in toxic material or oxygen-free water being brought to the surface

f Interval seiche movement in which a toxic or low DO hypolimnion flows up into a bay or bayou for a limited period of time, and later returns to normal level

2 Fish kills in rivers below high dams immediately following the opening of a gate permitting hypolimnionic water to flow down the stream (as in TVA region)

VI CASE HISTORY

A The Lower Mississippi Endrin kill is an excellent example of the investigation of a major fish kill. Bartsch and Ingram give the following summary (See Table 1).

TABLE 1

ELEMENTS OF INVESTIGATIONS

I	Examination of usual environmental factors
II	Elimination of parasites, bacterial or viral diseases and botulism as causes of mortalities*
III	Considerations of toxic substances: Examination and prognostication of symptoms of dying fish. Autopsy, including:
	Haematocrits and white cell counts
	Kidney tissue study
	Brain tissue assay for organic phosphorus insecticide
	Tissue analysis for 19 potentially toxic metals
	Gas chromatographic analysis of tissues, including blood, for chlorinated hydrocarbon insecticides
IV	Explorations for toxic substances:
	Bioassay with Mississippi River water
	Bioassay with extracts from river bottom mud
	Bioassay with tissue extracts from fish dying in river water and bottom mud extracts
	Bioassay with endrin to compare symptoms and tissue extract analyses with those of dying fish in all bioassays
V	Intensive chemical analysis for pesticides in the natural environment, experimental environment, river fish, and experimental animals
VI	Surveillance of surface waters for geographic range and intensity of pesticide contamination
VII	Correlation and interrelation of findings

* The investigator should be aware of the fact that apparently healthy fish may be harboring pathogenic bacteria in their bloodstreams (see Bullock and Snieszko). Thus there may be several factors involved in fish mortalities, all of which may obscure the primary cause or causes.

The investigation was designed to consider and eliminate potential fish kill possibilities that were not involved and come to a point focus on the real cause. It was found that the massive kills were not caused by disease, heavy metals, organic phosphorus compounds, lack of dissolved oxygen or unsuitable pH. Blood of dying river fish was found to have concentrations of endrin equal to or greater than laboratory fish killed with this pesticide, while living fish had lesser concentrations. Symptoms of both groups of dying fish were identical. It was concluded from all data obtained that these fish kills were caused by endrin poisoning.

B Recent investigations in Tennessee have shown that the leaking of small amounts of very toxic chemicals from spent pesticide-containing barrels used as floats for piers and diving rafts in lakes and reservoirs can produce extensive fish kills. The particular compound used to control slime growth in manufacturing processes, contained two primary chemicals in solution (phenylmercuric acetate and 2, 4, 6-trichlorophenol). The former compound which breaks down to form diphenylmercury was found to be more toxic to aquatic life than the latter.

REFERENCES

1 American Public Health Association, Inc. Standard Methods for the Examination of Water and Wastewater. Section 231 Bioassay, Examination of Polluted Waters, Wastewaters, Effluents, Bottom Sediments, and Sludges. Thirteenth Edition. New York. 1971.

2 Bartsch, A.F. and Ingram, William N. Biological Analysis of Water Pollution in North America. International Verein Limnol. 16:786-800. 1966.

3 Bullock, G.L. and Snieszko, S.F. Bacteria in Blood and Kidney of Apparently Healthy Hatchery Trout. Trans. American Fisheries Society 98(2):268-271. 1969.

4 Burdick, G.E. Some Problems in the Determination of the Cause of Fish Kills. Biol. Prob. in Water Pollution. USPHS Pub. No. 999-WP-25. pp. 289-292. 1965.

5 Fish Kills Caused by Pollution in 1970. 11th Annual Report 21 p. 1972.

6 Mount, Donald I. and Putnicki, George J. Summary Report of the 1963 Mississippi River Fish Kill Investigation, 31st North American Wildlife and Natural Res. Conf. 11 pp. 1966.

7 Smith, L. L. Jr., et al. Procedures for Investigation of Fish Kills (A guide for field reconnaissance and data collection). ORSANCO, Cincinnati, OH. 24 pp. 1956.

8 Tennessee Valley Authority. Fish Kill in Boone Reservoir. TVA Water Quality Branch, Chattanooga TN. 61 pp. 1968.

9 Tennessee State Game and Fish Commission. Field Manual for Investigation of Pollution and Fish Kills. (USPHS WPD 3-0351-65 Grant) 71 pp. undated.

10 Willoughby, L. G. Salmon Disease in Windermere and the River Leven; The Fungal Aspect. Salmon and Trout Magazine. 186:124-130. 1969.

11 Muncy, Robert J. Observations on the Factors Involved with Fish Mortality as the Result of Dinoflagellate "Bloom" in a Freshwater Lake. Proc. 17th Ann. Conf. Southeastern Assoc. of Game & Fish Commissioners. pp. 218-222.

This outline was prepared by Jack Geckler, Research Aquatic Biologist, Fish Toxicology Activities, EPA, Newtown, OH 45244

Project Personnel Contacted:

a. Name________________

b. Means of Contact________________

c. Date & Time____________

1. **Reporting source**

a. Agency____

(1) Address______________________________________

(2) Phone (s)____________________________________

b. Individual____

(1) Address______________________________________

(2) Phone____

(3) Fish Kill Network__________yes________no

c. Other Contacts______________________________________

(1) Address______________________________________

(2) Phone____

(3) Fish Kill Network__________yes________no

2. **Data furnished by reporting source**

a. Location of Kill____________________________________

b. Dates of Kill________________Dying last observed____________

c. Kinds of organisms__________________________________

d. Approximate number killed____________________________

e. Cause of kill (if known)_____________________________

f. Suspected causative sources_________________________

g. Measures taken___________________________________

h. Other Agencies contacted___________________________

(1) Date and Time_______________________________

3. **Action requested**

a. Field investigations_______________________________

b. Laboratory analysis_______________________________

4. **Assistance to Project**

a. Provided by____________________________________

b. Personnel______________________________________

c. Equipment______________________________________

d. Transportation facilities________________

BACTERIOLOGICAL INDICATORS OF WATER POLLUTION

Part 1. General Concepts

I INTRODUCTION

A Bacterial Indication of Pollution

1 In the broadest sense, a bacterial indicator of pollution is any organism which, by its presence, would demonstrate that pollution has occurred, and often suggest the source of the pollution.

2 In a more restrictive sense, bacterial indicators of pollution are associated primarily with demonstration of contamination of water, originating from excreta of warm-blooded animals (including man, domestic and wild animals, and birds).

B Implications of Pollution of Intestinal Origin

1 Intestinal wastes from warm-blooded animals regularly include a wide variety of genera and species of bacteria. Among these the coliform group may be listed, and species of the genera *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Proteus*, *Pseudomonas*, certain spore-forming bacteria, and others.

2 In addition, many kinds of pathogenic bacteria and other microorganisms may be released in wastes on an intermittent basis, varying with the geographic area, state of community health, nature and degree of waste treatment, and other factors. These may include the following:

a Bacteria: Species of *Salmonella*, *Shigella*, *Leptospira*, *Brucella*, *Mycobacterium*, and *Vibrio comma*.

b Viruses: A wide variety, including that of infectious hepatitis, Polioviruses, Coxsackie virus, ECHO viruses (enteric cytopathogenic human orphan -- "viruses in search of a disease"), and unspecified viruses postulated to account for outbreaks of diarrheal and upper respiratory diseases of unknown etiology, apparently infective by the water-borne route.

c Protozoa: *Endamoeba histolytica*

3 As routinely practiced, bacterial evidence of water pollution is a test for the presence and numbers of bacteria in wastes which, by their presence, indicate that intestinal pollution has occurred. In this context, indicator groups discussed in subsequent parts of this outline are as follows:

a Coliform group and certain subgroupings

b Fecal streptococci and certain subgroupings

c Miscellaneous indicators of pollution

4 Evidence of water contamination by intestinal wastes of warm-blooded animals is regarded as evidence of health hazard in the water being tested.

II PROPERTIES OF AN IDEAL INDICATOR OF POLLUTION

A An "ideal" bacterial indicator of pollution should:

1 Be applicable in all types of water

2 Always be present in water when pathogenic bacterial constituents of fecal contamination are present. Ramifications of this include --

  a Its density should have some direct relationship to the degree of fecal pollution.

  b It should have greater survival time in water than enteric pathogens.

  c It should disappear rapidly from water following the disappearance of pathogens, either through natural or man-made processes.

  d It always should be absent in a bacteriologically safe water.

3 Lend itself to routine quantitative testing procedures without interference or confusion of results due to extraneous bacteria

4 Be harmless to man and other animals

B In all probability, an "ideal" bacterial indicator does not exist. The discussion of bacterial indicators of pollution in the following parts of this outline include consideration of the merits and limitations of each group, with their applications in evaluating bacterial quality of water.

III APPLICATIONS OF TESTS FOR POLLUTION INDICATORS

A Tests for Compliance with Bacterial Water Quality Standards

1 Potability tests on drinking water to meet Interstate Quarantine or other standards of regulatory agencies.

2 Determination of bacterial quality of environmental water for which quality standards may exist, such as shellfish waters, recreational waters, water resources for municipal or other supplies.

3 Tests for compliance with established standards in cases involving the protection or prosecution of municipalities, industries, etc.

B Treatment Plant Process Control

1 Water treatment plants

2 Wastewater treatment plants

C Water Quality Surveys

1 Determination of intestinal pollution in surface water to determine type and extent of treatment required for compliance with standards

2 Tracing sources of pollution

3 Determination of effects on bacterial flora, due to addition of organic or other wastes

D Special Studies, such as

1 Tracing sources of intestinal pathogens in epidemiological investigations

2 Investigations of problems due to the Sphaerotilus group

3 Investigations of bacterial interference to certain industrial processes, with respect to such organisms as Pseudomonas, Achromobacter, or others

IV SANITARY SURVEY

The laboratory bacteriologist is not alone in evaluation of indication of water pollution of intestinal origin. On-site study (Sanitary Survey) of the aquatic environment and adjacent areas, by a qualified person, is a necessary collateral study with the laboratory work and frequently will reveal information regarding potential bacteriological hazard which may or may not be demonstrated through laboratory findings from a single sample or short series of samples.

Part 2. The Coliform Group and Its Constituents

I ORIGINS AND DEFINITION

A Background

1 In 1885, Escherich, a pioneer bacteriologist, recovered certain bacteria from human feces, which he found in such numbers and consistency as to lead him to term these organisms "the characteristic organism of human feces."

He named these organisms Bacterium coli-commune and B. lactis aerogenes. In 1895, another bacteriologist, Migula, renamed B. coli commune as Escherichia coli, which today is the official name for the type species.

2 Later work has substantiated much of the original concept of Escherich, but has shown that the above species are in fact a heterogeneous complex of bacterial species and species variants.

a This heterogeneous group occurs not only in human feces but representatives also are to be found in many environmental media, including sewage, surface freshwaters of all categories, in and on soils, vegetation, etc.

b The group may be subdivided into various categories on the basis of numerous biochemical and other differential tests that may be applied.

B Composition of the Coliform Group

1 Current definition

As defined in "Standard Methods for the Examination of Water and Wastewater" (12th ed): "The coliform group includes all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35° C."

2 The term "coliforms" or "coliform group" is an inclusive one, including the following bacteria which may meet the definition above:

a Escherichia coli, E. aurescens, E. freundii, E. intermedia

b Aerobacter aerogenes, A. cloacae

c Biochemical intermediates between the genera Escherichia and Aerobacter

3 There is no provision in the definition of coliform bacteria for "atypical" or "aberrant" coliform strains.

a An individual strain of any of the above species may fail to meet one of the criteria of the coliform group.

b Such an organism, by definition, is not a member of the coliform group, even though a taxonomic bacteriologist may be perfectly correct in classifying the strain in one of the above species.

II SUBDIVISION OF COLIFORMS INTO "FECAL" AND "NONFECAL" CATEGORIES

A Need

Single-test differentiations between coliforms of "fecal" origin and those of "nonfecal" origin are based on the assumption that typical E. coli and closely related strains are of fecal origin while A. aerogenes and its close relatives are not of direct fecal origin. (The latter assumption is not fully borne out by investigations at this Center. See Table 1, IMViC Type --++). A number of single differential tests have been proposed to differentiate between "fecal" and "nonfecal" coliforms.

Without discussion of their relative merits, several may be cited here:

B Types of Single-Test Differentiation

1 Determination of gas ratio

Fermentation of glucose by E. coli results in gas production, with hydrogen and carbon dioxide being produced in equal amounts. Fermentation of glucose by A. aerogenes results in generation of twice as much carbon dioxide as hydrogen.

Further studies suggested absolute correlation between H_2/CO_2 ratios and the terminal pH resulting from glucose fermentation. This led to the substitution of the methyl red test.

2 Methyl red test

Glucose fermentation by E. coli typically results in a culture medium having terminal pH in the range 4.2 - 4.6 (red color a positive test with the addition of methyl red indicator). A. aerogenes typically results in a culture medium having pH 5.6 or greater (yellow color, a negative test).

3 Indole

When tryptophane, an amino acid, is incorporated in a nutrient broth, typical E. coli strains are capable of producing indole (positive test) among the end products, whereas A. aerogenes does not (negative test).

In reviewing technical literature, the worker should be alert to the method used to detect indole formation, as the results may be greatly influenced by the analytical procedure.

4 Voges-Proskauer test (acetylmethyl carbinol test)

The test is for detection of acetylmethyl carbinol, a derivative of 2, 3, butyleneglycol, as a result of glucose fermentation in the presence of peptone. A. aerogenes produces this end product (positive test) whereas E. coli gives a negative test.

a Experience with coliform cultures giving a positive test has shown a loss of this ability with storage on laboratory media for 6 months to 2½ years, in 20 - 25% of cultures (105 out of 458 cultures).

b Some workers consider that all coliform bacteria produce acetylmethyl carbinol in glucose metabolism. These workers regard acetylmethyl carbinol-negative cultures as those which have enzyme systems capable of further degradation of acetylmethyl carbinol to other end products which do not give a positive test with the analytical procedure. Cultures giving a positive test for acetylmethyl carbinol lack this enzyme system.

c This reasoning leads to a hypothesis (not experimentally proven) that the change of reaction noted in certain cultures in 4.a above is due to the activation of a latent enzyme system.

5 Citrate utilization

Cultures of E. coli are unable to use the carbon of citrates (negative test) in their metabolism, whereas cultures of A. aerogenes are capable of using the carbon of citrates in their metabolism (positive test).

Some workers (using Simmons Citrate Agar) incorporate a pH indicator (brom thymol blue) in the culture medium in order to demonstrate the typical alkaline reaction (pH 8.4 - 9.0) resulting with citrate utilization.

6 Elevated temperature (Eijkman) test

a The test is based on evidence that E. coli and other coliforms of fecal origin are capable of growing and fermenting carbohydrates (glucose or lactose) at temperatures significantly higher than the body temperature of warm-blooded animals. Organisms not associated with direct fecal origin would give a negative test result, through their inability to grow at the elevated temperature.

b While many media and techniques have been proposed, EC Broth, a medium developed by Perry and Hajna, used as a confirmatory medium for 24 hours at 44.5 ± 0.2° C are the current recommended medium and method of choice. While the "EC" terminology of the medium suggests "E. coli" the worker should not regard this as a specific procedure for isolation of E. coli.

c A similar medium, Boric Acid Lactose Broth, has been developed by Levine and his associates. This medium gives results virtually identical with those obtained from EC Broth, but requires 48 hours of incubation.

d Elevation temperature tests require incubation in a water bath. Standard Methods 13th Ed. requires this temperature to be 44.5 ± 0.2° C. Various workers have urged use of temperatures ranging between 43.0° C and 46.0° C. Most of these recommendations have provided a tolerance of ± 0.5° C from the recommended levels. However, some workers, notably in the Shellfish Program of the Public Health Service, stipulate a temperature of 44.5 ± 0.2° C. This requires use of a water bath with forced circulation to maintain this close tolerance. This tolerance range has been instituted in the 13th Edition of Standard Methods and the laboratory worker should conform to these new limits.

e The reliability of elevated temperature tests is influenced by the time required for the newly-inoculated cultures to reach the designated incubation temperature. Critical workers insist on placement of the cultures in the water bath within 30 minutes, at most, after inoculation.

7 Other tests

Numerous other tests for differentiation between coliforms of fecal vs. nonfecal origin have been proposed. Current studies suggest little promise for the following tests in this application: uric acid test, cellobiose fermentation, gelatin liquefaction, production of hydrogen sulfide, sucrose fermentation, and others.

C IMViC Classification

1 In 1938, Parr reported on a review of a literature survey on biochemical tests used to differentiate between coliforms of fecal vs. nonfecal origin. A summary follows:

Test	No. of times used for differentiation
Voges-Proskauer reaction	22
Methyl red test	20
Citrate utilization	20
Indole test	15
Uric acid test	6
Cellobiose fermentation	4
Gelatin liquefaction	3
Eijkman test	2
Hydrogen sulfide production	1
Sucrose fermentation	1
a-Methyl-d-glucoside fermentation	1

2 Based on this summary and on his own studies, Parr recommended utilization of a combination of tests, the indole, methyl red, Voges-Proskauer, and the citrate utilization tests for this differentiation. This series of reactions is designated by the mnemonic "IMViC". Using this scheme, any coliform culture can be described by an "IMViC Code" according to the reactions for each culture. Thus, a typical culture of E. coli would have a code ++--, and a typical A. aerogenes culture would have a code --++.

3 Groupings of coliforms into fecal, non-fecal, and intermediate groups, as shown in "Standard Methods for the Examination of Water and Wastewater" are shown at the bottom of this page.

D Need for Study of Multiple Cultures

All the systems used for differentiation between coliforms of fecal vs. those of nonfecal origin require isolation and study of numerous pure cultures. Many workers prefer to study at least 100 cultures from any environmental source before attempting to categorize the probable source of the coliforms.

III NATURAL DISTRIBUTION OF COLIFORM BACTERIA

A Sources of Background Information

Details of the voluminous background of technical information on coliform bacteria recovered from one or more environmental media are beyond the scope of this discussion. References 2, 3, and 4 of this outline are suggested routes of entry for workers seeking to explore this topic.

B Recent Studies on Coliform Distribution

1 Since 1960, workers at this Center have engaged in a continuing study of the natural distribution of coliform bacteria and an evaluation of procedures for differentiation between coliforms of fecal vs. probable nonfecal origin. Results of this work have special significance because:

a Rigid uniformity of laboratory methods have been applied throughout the series of studies

b Studies are based on massive numbers of cultures, far beyond any similar studies heretofore reported

Groupings of Coliforms into Fecal, Nonfecal and Intermediate Groups

Organism	Indole	Methyl red	Voges-Proskauer	Citrate
E. coli, Variety I	+	+	-	-
Variety II	-	+	-	-
E. freundii (Intermediates)				
Variety I	-	+	-	±
Variety II	+	+	-	+
A. aerogenes				
Variety I	-	-	+	±
Variety II	±	-	+	+

c A wider variety of environmental and biological sources is being studied than in any previous series of reports.

d All studies are based on freshly recovered pure culture isolates from the designated sources.

e All studies are based on cultures recovered from the widest feasible geographic range, collected at all seasons of the year. It is believed that no more representative series of studies has been made or is in progress.

2 Distribution of coliform types

Table 1 shows the consolidated results of coliform distributions from various biological and environmental sources.

a The results of these studies show a high order of correlation between known or probable fecal origin and the typical E. coli IMViC code (++--). On the other hand, human feces also includes numbers of A. aerogenes and other IMViC types, which some regard as "nonfecal" segments of the coliform group. (Figure 1)

b The majority of coliforms attributable to excretal origin tend to be limited to a relatively small number of the possible IMViC codes; on the other hand, coliform bacteria recovered from undisturbed soil, vegetation, and insect life represent a wider range of IMViC codes than fecal sources, without clear dominance of any one type. (Figure 2)

c The most prominant IMViC code from nonfecal sources is the intermediate type, -+-+, which accounts for almost half the coliform cultures recovered from soils, and a high percentage of those recovered from vegetation and from insects. It would appear that if any coliform segment could be termed a "soil type" it would be IMViC code -+-+.

d It should not be surprising that cultures of typical E. coli are recovered in relatively smaller numbers from sources judged, on the basis of sanitary survey, to be unpolluted. There is no known way to exclude the influence of limited fecal pollution from small animals and birds in such environments.

e The distribution of coliform types from human sources should be regarded as a representative value for large numbers of sources. Investigations have shown that there can be large differences in the distribution of IMViC types from person to person, or even from an individual.

3 Differentiation between coliforms of fecal vs. nonfecal origin

Table 2 is a summary of findings of SEC workers, based on a number of different criteria for differentiating between coliforms of fecal origin and those from other sources.

a IMViC type ++-- is a measurement of E. coli, Vairety I, and appears to give reasonably good correlation between known or highly probable fecal origin and doubtful fecal origin.

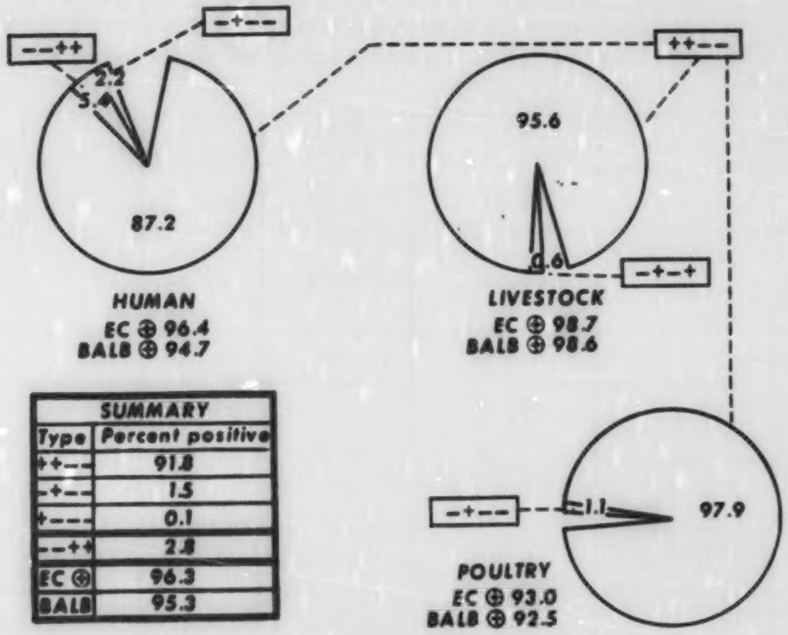
b The combination of IMViC types, ++--, +---, and -+--, gives improved identification of probable fecal origin, and appears also to exclude most of the coliforms not found in excreta of warm-blooded animals in large numbers.

c While the indole, methyl red, Voges Proskauer, and citrate utilization tests, each used alone, appear to give useful answers when applied only to samples of known pollution from fecal sources, the interpretation is not as clear when applied to coliforms from sources believed to be remote from direct fecal pollution.

Table 1. COLIFORM DISTRIBUTION BY IMViC TYPES AND ELEVATED TEMPERATURE TEST FROM ENVIRONMENTAL AND BIOLOGICAL SOURCES

IMViC type	Vegetation		Insects		Soil				Fecal sources				Poultry	
					Undisturbed		Polluted		Human		Livestock			
	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total
++--	128	10.6	134	12.4	131	5.6	536	80.6	3932	87.2	2237	95.6	1857	97.9
--++	237	19.7	113	10.4	443	18.8	13	2.0	245	5.4	0	<0.1	1	0.1
-+--	23	1.9	0	<0.1	78	3.3	1	0.2	99	2.2	14	0.6	20	1.1
+++-	2	0.2	0	<0.1	7	0.3	0	<0.1	106	2.4	59	2.5	0	<0.1
-+-+	168	14.0	332	30.6	1131	48.1	87	13.0	50	1.1	1	<0.1	5	0.3
++-+	116	9.6	118	10.9	87	3.7	22	3.3	35	0.8	27	1.2	11	0.6
-+++	32	2.7	28	2.6	181	7.7	5	0.7	21	0.5	0	<0.1	0	<0.1
++++	291	24.2	254	23.4	159	6.8	0	<0.1	6	0.1	0	<0.1	0	<0.1
+-++	88	7.3	46	4.2	67	2.9	0	<0.1	14	0.2	0	<0.1	0	<0.1
---+	87	7.2	42	3.9	4	0.2	1	0.2	2	<0.1	0	<0.1	0	<0.1
-++-	5	0.4	0	<0.1	1	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
--+-	19	1.6	0	<0.1	53	2.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+-+-	2	0.2	0	<0.1	6	0.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+--+	5	0.4	8	0.7	0	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+---	0	<0.1	9	0.8	0	<0.1	0	<0.1	2	<0.1	0	<0.1	2	<0.1
Total	1203		1084		2348		665		4512		2339		1896	
No. EC +	169*		162*		216		551		4349		2309		1765	
% EC +	14.1*		14.9*		9.2		82.9		96.4		98.7		93.0	

Vegetation	Insects
*120 of these were ++--, 15 --++, 11 -+--	*129 of these were ++--, 27 -+-+, 5 ++-+



SUMMARY	
Type	Percent positive
++––	91.8
–+––	1.5
+–––	0.1
––++	2.8
EC ⊕	96.3
BALB	95.3

FIGURE 1

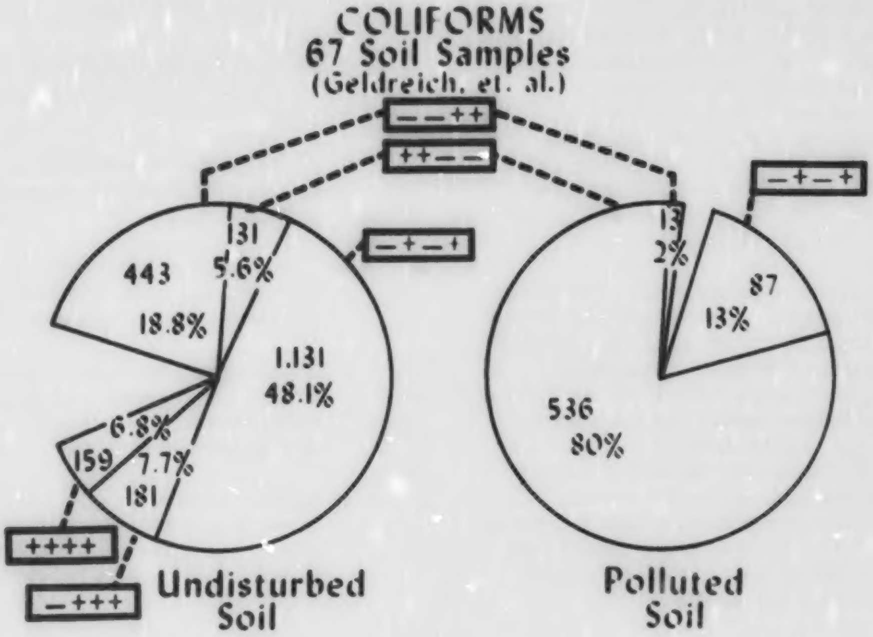


FIGURE 2

Table 2. COMPARISON OF COLIFORM STRAINS ISOLATED FROM WARM-BLOODED ANIMAL FECES, FROM UNPOLLUTED SOILS AND POLLUTED SOILS WITH USE OF THE IMVIC REACTIONS AND THE ELEVATED TEMPERATURE TEST IN EC MEDIUM AT 44.5° C (±0.5°) (12th ed. 1965; Standard Methods for the Examination of Water and Wastewater)

Test	Warm-blooded animal feces	Soil: Unpolluted	Soil: Polluted	Vege- tation	Insects
+ + - -	91.8%	5.6%	80.6%	10.6%	12.4%
+ + - -, + - - - and - + - -	93.3%	8.9%	80.7%	12.5%	13.2%
Indole positive	94.0%	19.4%	82.7%	52.5%	52.4%
Methyl red positive	96.9%	75.6%	97.9%	63.6%	79.9%
Voges-Proskauer positive	5.1%	40.7%	97.3%	56.3%	40.6%
Citrate utilizers	3.6%	88.2%	19.2%	85.1%	86.7%
Elevated temperature (EC) positive	96.4%	9.2%	82.9%	14.1%	14.9%
Number of cultures studied	8,747	2,348	665	1,203	1,084

Total Pure Cultures Studied: 14,047

d The elevated temperature test gives excellent correlation with samples of known or highly probable fecal origin. The presence of smaller, but demonstrable, percentages of such organisms in environmental sources not interpreted as being polluted could be attributed largely to the warm-blooded wildlife in the area, including birds, rodents, and other small mammals.

e The elevated temperature test yields results equal to those obtained from the total IMViC code. It has marked advantages in speed, ease and simplicity of performance, and yields quantitative results for each water sample. Therefore, it is regarded as the method of choice for differentiation between coliforms of probable direct fecal origin and those which may have become established in the bacterial flora of the aquatic or terrestrial habitat.

IV EVALUATION OF COLIFORMS AS POLLUTION INDICATORS

A The Coliform Group as a Whole

1 Merits

a The absence of coliform bacteria is evidence of a bacteriologically safe water.

b The density of coliforms is roughly proportional to the amount of excretal pollution present.

c If pathogenic bacteria of intestinal origin are present, coliform bacteria also are present, in much greater numbers.

d Coliforms are always present in the intestines of humans and other warm-blooded animals, and are eliminated in large numbers in fecal wastes.

e Coliforms are more persistent in the aquatic environment than are pathogenic bacteria of intestinal origin.

f Coliforms are generally harmless to humans and can be determined quantitatively by routine laboratory procedures.

2 Limitations

a Some of the constituents of the coliform group have a wide environmental distribution in addition to their occurrence in the intestines of warm-blooded animals.

b Some strains of the coliform group may multiply in certain polluted waters ("aftergrowth"), of high nutritive values thereby adding to the difficulty of evaluating a pollution situation in the aquatic environment. Members of the A. aerogenes section of the coliform are commonly involved in this kind of problem.

c Because of occasional aftergrowth problems, the age of the pollution may be difficult to evaluate under some circumstances.

d Tests for coliforms are subject to interferences due to other kinds of bacteria. False negative results sometimes occur when species of Pseudomonas are present. False positive results sometimes occur when two or more kinds of non-coliforms produce gas from lactose, when neither can do so alone (synergism).

B The Fecal Coliform Component of the Coliform Group (as determined by elevated temperature test)

1 Merits

a The majority (over 95% of the coliform bacteria from intestines of warm-blooded animals grow at the elevated temperature.

b These organisms are of relatively infrequent occurrence except in association with fecal pollution.

c Survival of the fecal coliform group is shorter in environmental waters than for the coliform group as whole. It follows, then, that high densities of fecal coliforms is indicative of relatively recent pollution.

d Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. In certain high-carbohydrate wastes, such as from the sugar beet refineries, exceptions have been noted.

2 Limitations

a Feces from warm-blooded animals include some (though proportionately low) numbers of coliforms which do not yield a positive fecal coliform test when the elevated temperature test is used as the criterion of differentiation. These organisms are E. coli varieties by present taxonomic classification.

b There is at present no established and consistent correlation between ratios of total coliforms/fecal coliforms in interpreting sanitary quality of environmental waters.

In domestic sewage, the fecal coliform density commonly is greater than 90% of the total coliform density. In environmental waters relatively free from recent pollution, the fecal coliform density may range from 10-30% of the total coliforms. There are, however, too many variables relating to water-borne wastes and surface water runoff to permit sweeping generalization on the numerical relationships between fecal- and total coliforms.

c At this time, evaluations are underway regarding the survival of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria. In recent pollution studies, species of Salmonella have been found in the presence of 220 fecal coliforms per 100 ml (Spino), and 110 fecal coliforms per 100 ml (Brezenski, Raritan Bay Project).

V APPLICATIONS OF COLIFORM TESTS

A Current Status in Official Tests

1 The coliform group is designated, in "Standard Methods for the Examination of Water and Wastewater" (13th ed., 1971), through the Completed Test MPN procedure as the official test for bacteriological potability of water. The Confirmed Test MPN procedure is accepted where it has been demonstrated, through comparative tests, to yield results equivalent to the Completed Test. The membrane filter method also is accepted for examination of waters subject to interstate regulation.

2 The 12th edition of Standard Methods has introduced a standard test for fecal coliform bacteria. It is emphasized that this is to be used in pollution studies, and does not apply to the evaluation of water for potability. This procedure has been carried to the 13th Edition.

B Applications

1 Tests for the coliform group as a whole are used in official tests to comply with interstate drinking water standards, state standards for shellfish waters, and in most, if not all, cases where bacterial standards of water quality have been established for such use as in recreational or bathing waters, water supplies, or industrial supplies. Laboratory personnel should be aware of possible implementation of the fecal coliform group as the official test for recreational and bathing waters.

2 The fecal coliform test has application in water quality surveys, as an adjunct to determination of total coliform density. The fecal coliform test is being used increasingly in all water quality surveys.

3 It is emphasized that no responsible worker advocates substitution of a fecal coliform test for total coliforms in evaluating drinking water quality.

Part 3. The Fecal Streptococci

I DEFINITIONS

A A fecal streptococcus as used in this discussion refers to any streptococcus commonly found in significant numbers in the feces of human or other warm-blooded animals. Other terms used are "enterococci" or "Group D streptococci."

B Enterococci are characterized by specific taxonomic biochemistry. Serological procedures differentiate the Group D streptococci from the various groups. Although they overlap, the three groups, fecal streptococcus, enterococcus, and Group D streptococcus, are not synonymous. Because our emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term and will include the enterococcus as well as other groups.

C A rigid definition of the fecal streptococcus group is not possible with our present knowledge. The British Ministry of Health (1956) defines the organisms as "Gram-positive" cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45° C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria." However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water."

D Some workers consider that growth at 45° C and multiplication in 40% bile are the most significant indications of fecal origin of streptococci.

E
1 The first group, the Pyogenic Group, not growing at 10° C or 45° C, pathogenic for man and animals, causes total lysing of red blood cells, giving them the common name of beta hemolytic streptococci. These organisms are not considered as belonging to the fecal streptococci.

2 A second group, or Viridans group, not growing at 10° C, growth at 45° C, is an intermediate group, some members resembling group 1 and others.

3 The third group, the Enterococcus Group, growing at 10° C and 45° C, and typically exhibit vigorous biochemical reactions. Members of these groups (2 and 3) are considered to be fecal streptococci.

4 The fourth, the Lactic Group, growing at 10° C and not at 45° C, is a common harmless inhabitant of plants and dairy products and bear no relationship to the definition of a fecal streptococcus.

Examples of specific strains or species representative of each group are:

a *Streptococcus pyogenes*, cause of scarlet fever

b *Streptococcus bovis*, from cow manure

c *Streptococcus fecalis*, from human feces

d *Streptococcus lactis*, from cheese

F For special purposes, strains of individual fecal streptocci are identified by streptocci are identified by biochemical tests. The techniques are discussed elsewhere. Strains or species likely to be fecal streptococci are: *Streptococcus fecalis*, *S. fecalis* variety *liquefaciens*, *S. fecalis* variety *zymogenes*, *S. durans*, *S. bovis*, *S. equinus*, *S. salivarius*, and *S. fecalis* biotypes usually growing at 10° C and 45° C.

II HISTORY

From their discovery to the present time the fecal streptococci appear characteristic of fecal pollution, being consistently present in both the feces of all warm-blooded animals and in the environment associated with animal discharges. As early as 1910 fecal streptococci were proposed as indicators to the Metropolitan Water Board of London. However, little progress resulted in the United States until improved methods of detection and enumeration appeared after World War II.

Renewed interest in the group as indicators began with the introduction of azide dextrose broth in 1950, (Mallmann & Seligmann, 1950). The method which is in the current edition of Standard Methods appeared soon after. (Litsky, et al. 1955).

With the advent of improved methods for detection and enumeration of fecal streptococci, a significant body of technical literature has appeared.

III SIGNIFICANCE OF FECAL STREPTOCOCCAL INDICATORS

A The presence of fecal streptococci indicates the presence of warm-blooded animal pollution. Many types of wild life may harbor fecal streptococci. One study showed 71% of various mammalian species yielding enterococci. Similarly, reptiles showed 85% incidence. However, the common rodents yielded few and sporadic isolations. Wild birds also showed few individuals with enterococci. (Mundt 1963). Insects may be only accidental mechanical carriers of fecal streptococci. However, Geldreich, et al (1964) found high numbers in some insects.

B Evidence indicates that fecal streptococci do not occur in pure water or virgin soil.

In one study, Medrek and Litsky (1960) looked specifically for occurrence of enterococci in undisturbed or virgin soil. Modern methods showed 8 out of 369 samples with enterococci. With only one exception, the presence of enterococci in a given soil sample was associated with the presence of coliforms. The presence of indicators in a given soil sample is considered indicative of chance pollution of the sampling area by animal or bird droppings or drainage.

C Fecal streptococci do not multiply in water. A recent report is illustrative (Morris and Weaver, 1954). Duplicate samples were taken of 52 polluted Kentucky wells and examined before and after storage for 24 hours. While the coliform numbers increased, decreased or changed little, the streptococcal numbers never increased.

Other experiments on a variety of Louisiana surface water gave same results for streptococcal numbers. Fecal streptococci enter a water, survive for a period of time and die without multiplying.

D On the basis of laboratory data, the fecal streptococci could be expected to be more resistant to electrolytes like salt. If true, the group would have special value for salt water investigation. Further research is needed to determine this point.

E The fecal streptococci are not considered pathogenic. However, such strains as S. fecalis have been known to occur with endocarditis and other human infections. S. fecalis var. zymogenes, a beta hemolytic streptococcus normally inhabiting the human intestine, is also nonpathogenic.

The fecal streptococci are alleged causes of food-borne infections. However, the circumstantial evidence has not been supported by intensive studies.

IV LIMITATIONS

A Knowledge of relative survival times of fecal streptococci and pathogens is needed. Comparable results with many types of pathogens under many environmental conditions are needed. Insufficient time has elapsed since adequate media have been developed for evidence to accumulate. The coliforms have over 60 years of accumulated validation for their use. The fecal streptococci have had only about 15 years of serious consideration. A few comparisons exist, however.

When sewage is added to the soil, the coliforms persist for long periods. Fecal streptococci disappear rapidly. Typhoid bacilli, added experimentally with sewage, persist less time than the fecal streptococci. However, mineral-organic content of the receiving soil has much influence on persistence so that only relative rates may be determined. (Mallman and Litsky, 1950).

B Although the enterococci are considered as having their origin, in the intestines of warm-blooded animals, other sources may exist. The resistance and tolerance of these streptococci, together with their low minimum and high maximum limits of growth may not only enable them to survive but also to grow under diverse conditions of nature. Reports have appeared that growth occurs in food products, fresh plants and silage. For example, some investigations (Mundt, 1962) show that fecal streptococci of the S. fecalis and the S. liquefaciens types occur commonly on plants. However, Geldreich et al, 1963 concluded after an exhaustive study of vegetation that indicator contribution was negligible.

V FECAL COLIFORM-FECAL STREPTOCOCCUS RELATIONSHIPS

Because coliforms may derive from non-fecal sources, the fecal coliforms are a more reliable standard of comparison with streptococcal densities. Even so, variation in known ratios is marked.

A Variation may arise from different methods, different species, different individuals, and different geographic locations.

Table 3. ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS*

		Average indicator density per gram of feces		Average contribution per capita per 24 hr		
Animals	Avg wt of Feces/24 hr, wet wt, g	Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	Ratio FC/FS
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

*Publication WP-20-3, P. 102

B Species differences are the main cause of different fecal coliform-fecal streptococci ratios. Table 3 compares fecal streptococcus and fecal coliform counts for different species. Even though individuals vary widely, masses of individuals in a species have characteristic proportion of indicators. In intestinal wastes of human origin, the ratio of number of fecal coliforms to number of fecal streptococci tends to be considerably greater than two-to-one. In comparison, when such ratios are determined for intestinal wastes from non-human animal sources, the values tend to be markedly less than one-to-one.

Current interpretative use of this ratio is as follows:

Domestic Wastes (Predominately Human)	- > 4.0
Animal Wastes	- < 0.7

C Determination of specific strains of fecal streptococci sometimes is useful. Besides ratio of indicators, the specific types of fecal streptococci are more typical of some animals. Humans carry 80% or over of S. fecalis group, and characteristically the S. salivarius-mitis group. Cows and horses carry a majority of S. bovis and S. equinus groups. Other farm animals have mixtures of appreciable numbers of several groups. Fowl, however, resemble humans in their streptococcal flora. The S. salivarius group, besides being uniquely human, dies rapidly in water, giving an index of recentness of pollution.

VI INTERPRETATION

The presence of fecal streptococci in untreated water indicates the presence of fecal pollution by warm-blooded animals.

In samples where the source and significance of the coliform group has been questioned, the presence of the streptococcus group should be interpreted as indicating at least a portion of the coliform group were derived from fecal sources.

Because of the uncertainties in die-off rates, the absence of fecal streptococci does not necessarily mean that the water is bacteriologically safe.

Part 4. Other Bacterial Indicators of Pollution

I TOTAL BACTERIAL COUNTS

A Historical

1 The early studies of Robert Koch led him to develop tentative standards of water quality based on a limitation of not more than 100 bacterial colonies per ml on a gelatin plating medium incubated 3 days at 20° C.

2 Later developments led to inoculation of samples on duplicate plating media, with one set incubated at 37° C and the other at 20° C.

a Results were used to develop a ratio between the 37° C counts and the 20° C counts.

b Waters having a predominant count at 37° C were regarded as being of probable sanitary significance, while those giving predominant counts at 20° C were considered to be of probable soil origin, or natural inhabitants of the water being examined.

B Groups Tested

There is no such thing as "total" bacterial count in terms of a laboratory determination.

1 Direct microscopic counts do not differentiate between living and dead cells.

2 Plate counting methods enumerate only the bacteria which are capable of using the culture medium provided, under the temperature and other growth conditions used as a standard procedure. No one culture medium and set of growth conditions can provide, simultaneously, an acceptable environment for all the heterogeneous, often conflicting, requirements of the total range of bacteria which may be recovered from waters.

C Utilization of Total Counts

1 Total bacterial counts, using plating methods, are useful for:

a Detection of changes in the bacterial composition of a water source

b Process control procedures in treatment plant operations

c Determination of sanitary conditions in plant equipment or distributional systems

2 Serious limitations in total bacterial counts exist because:

a No information is given regarding possible or probable fecal origin of bacterial changes. Large numbers of bacteria can sometimes be cultivated from waters known to be free of fecal pollution.

b No information of any kind is given about the species of bacteria cultivated.

c There is no differentiation between harmless or potentially dangerous forms.

3 Status of total counts

a There is no total bacterial count standard for any of the following:

Interstate Quarantine Drinking Water Standards

PHS regulations for water potability (as shown in "Standard Methods" Public Health Service Drinking Water Standards of 1962.)

b The most widely used current application of total bacterial counts in water bacteriology today is in water treatment plants, where some workers use standard plate counts for process control and for determination of the bacterial quality of distribution systems and equipment.

c Total bacterial counts are not used in PHS water quality studies, though extensively used until the 1940's.

B Spore-Forming Bacteria (Clostridium perfringens, or C. welchii)

1 Distribution

This is one of the most widely distributed species of bacteria. It is regularly present in the intestinal tract of warm-blooded animals.

2 Nature of organism

C. perfringens is a Gram-positive, spore-forming rod. The spores cause a distinct swelling of the cell when formed. The organism is extremely active in fermentation of carbohydrates, and produces the well-known "stormy fermentation" of milk.

3 Status

The organism, when present, indicates that pollution has occurred at some time. However, because of the extremely extended viability of the spores, it is impossible to obtain even an approximation of the recency of pollution based only on the presence of C. perfringens.

The presence of the organism does not necessarily indicate an unsafe water.

C Tests for Pathogenic Bacteria of Intestinal Origin

1 Groups considered include Salmonella sp, Shigella sp, Vibrio comma, Mycobacterium sp, Pasteurella sp, Leptospira sp, and others.

2 Merits of direct tests:

Demonstration of any pathogenic species would demonstrate an unsatisfactory water quality, hazardous to persons consuming or coming into contact with that water.

3 Limitations

a There is no available routine procedure for detection of the full range of pathogenic bacteria cited above.

b Quantitative methods are not available for routine application to any of the above.

c The intermittent release of these pathogens makes it impossible to regard water as safe, even in the absence of pathogens.

d After detection, the public already would have been exposed to the organism; thus, there is no built-in margin of safety, as exists with tests for the coliform group.

4 Applications

a In tracing the source of pathogenic bacteria in epidemiological investigations

b In special research projects

c In water quality studies concerned with enforcement actions against pollution, increasing attention is being given to the demonstration of enteric pathogenic bacteria in the presence of the bacterial indicators of pollution.

D Miscellaneous Indicators

It is beyond this discussion to explore the total range of microbiological indicators of pollution that have been proposed and investigated to some extent. Mention can be made, however, of consideration of tests for the following:

1 Bacteriophages specific for any of a number of kinds of bacteria

2 Serological procedures for detection of coliforms and other indicators; a certain amount of recent attention has been given to applications of fluorescent antibodies in such tests

3 Tests for Pseudomonas aeruginosa

4 Tests for viruses, which may persist in waters even longer than members of the coliform group.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, 12th ed., APHA, AWWA, WPCF. Published by American Public Health Association, 1790 Broadway, New York, N.Y. 1965.

2 Prescott, S.C., Winslow, C.E.A., and McCrady, M. Water Bacteriology. John Wiley & Sons, Inc. 1946.

3 Parr, L.W. Coliform Intermediates in Human Feces. Jour. Bact. 36:1. 1938.

4 Clark, H.F. and Kabler, P.W. The Physiology of the Coliform Group. Proceedings of the Rudolfs Research Conference on Principles and Applications in Aquatic Microbiology. 1963.

5 Geldreich, E.E., Bordner, R.H., Huff, C.B., Clark, H.F., and Kabler, P.W. Type Distribution of Coliform Bacteria in the Feces of Warm-Blooded Animals. JWPCF. 34:295-301. 1962.

6 Geldreich et al. The Fecal Coli-Aerogenes Flora of Soils from Various Geographic Areas. Journal of Applied Bacteriology 25:87-93. 1962.

7 Geldreich, E.E., Kenner, B.A., and Kabler, P.W. Occurrence of Coliforms, Fecal Coliforms, and Streptococci on Vegetation and Insects. Applied Microbiology. 12:63-69. 1964.

8 Kabler, P.W., Clark, H.F., and Geldreich, E.E. Sanitary Significance of Coliform and Fecal Coliform Organisms in Surface Water. Public Health Reports. 79:58-60. 1964.

9 Clark, H.F. and Kabler, P.W. Re-evaluation of the Significance of the Coliform Bacteria. Journal AWWA. 56:931-936. 1964.

10 Kenner, B.S., Clark, H.F., and Kabler, P.W. Fecal Streptococci. II. Quantification in Feces. Am. J. Public Health. 50:1553-59. 1960.

11 Litsky, W., Mallman, W.L., and Fifield, C.W. Comparison of MPN of Escherichia coli and Enterococci in River Water. Am. Jour. Public Health. 45:1949. 1955.

12 Medrek, T.F. and Litsky, W. Comparative Incidence of Coliform Bacteria and Enterococci in Undisturbed Soil. Applied Microbiology. 8:60-63. 1960.

13 Mallman, W.L., and Litsky, W. Survival of Selected Enteric Organisms in Various Types of Soil. Am. J. Public Health. 41:38-44. 1950.

14 Mallman, W.L., and Seligman, E.B., Jr. A Comparative Study of Media for Detection of Streptococci in Water and Sewage. Am. J. Public Health. 40:286-89. 1950.

15 Ministry of Health (London). The Bacterial Examination of Water Supplies. Reports on Public Health and Medical Subjects. 71:34.

16 Morris, W. and Weaver, R.H. Streptococci as Indices of Pollution in Well Water. Applied Microbiology. 2:282-285. 1954.

17 Mundt, J.O., Coggin, J.H., Jr., and Johnson, L.F. Growth of *Streptococcus fecalis* var. *liquefaciens* on Plants. Applied Microbiology. 10:552-555. 1962.

18 Geldreich, E.E. Sanitary Significance of Fecal Coliforms in the Environment. U.S. Department of the Interior. FWPCA Publ. WP-20-3. 1966.

This outline was prepared by H. L. Jeter, Director, National Training Center, OWP, EPA, Cincinnati, OH 45268.

BIOASSAY AND BIOMONITORING

I INTRODUCTION

A An assay is an evaluation.

B A bioassay is an evaluation in which living organisms provide the scale.

1 The scale or degree of response may be the rate of growth or decrease of a population, colony, or individual; a behaviorial, physiological, or reproductive response, or simply a live or no-live response.

2 All types of bioassays may have a role to play in water quality evaluation at one time or another.

3 The particular group of bioassays discussed below are those which contribute to the evaluation of the effects of liquid wastes on aquatic environments in which experimental organisms such as fish are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions for a stipulated period of time.

C Historical Highlights

1 Prior to 1940, there was little or no uniformity in performing or reporting bioassays of water pollutants.

2 By mid-forties, the need for a standardized technique was becoming painfully obvious.

a The Atlantic Refining Company privately published the first statement of what is today, basically, our standard method (Hart, Doudoroff, and Greenbank, 1945).

b This was refined and accorded wide (but not universal) industrial, academic, and governmental acceptance in 1951 (Doudoroff, et al, 1951).

3 This method was first developed for the use of fishes, but has been found adaptable to a wide variety of organisms.

D Other Types or Plans of Bioassay

1 Many other designs for the expression of toxicity have been devised such as those based on time-concentration curves. Each has its advantages and proponents, but the basic Standard Method design remains the most widely used.

a In situ exposure of experimental organisms in cages or live cars, at selected sites above and below a suspected point or pollution is an obvious and time tested procedure, but lacks the precision of laboratory tests. It has the advantages of popular appeal, and of expressing actual environmental conditions.

b The familiar BOD test is a bioassay of the organic content of water subject to biodegradation.

E Biomonitoring

Water quality surveillance or monitoring by means of observing biota can be considered from two aspects: field and laboratory. It differs from bioassay primarily in the objective: a bioassay is an attempt to determine a specific defined value or threshold, whereas a biomonitoring operation is an attempt to use living organisms to ascertain whether or not aquatic life is endangered.

1 Periodic biological field surveys, samples, or other observation may demonstrate recent excessive pollution for example.

2 Organisms in a series of flow-through tanks in a laboratory may demonstrate the occurrence of an unacceptable

increase in the toxicity of an effluent, without measuring "how much" or "what."

II THE STANDARD METHOD BIOASSAY

A Introduction

1 This procedure is intended for use by industrial and other laboratories.

2 Its objective is to evaluate the toxicity of wastes and other water pollutants to fish or other aquatic organisms.

3 Potential applications are numerous.

- a Dilution and/or treatment necessary to avoid acute toxic effects can be estimated.
- b The efficacy of an existing treatment can be tested.
- c The potential usefulness of a proposed treatment can be estimated.

4 The design of the test need not involve a chemical knowledge of the toxicant.

- a Synergism, antagonism, and other interactions of chemical components cannot always be anticipated, but are automatically included in the result.
- b All chemical and physical information available is, however, essential to the adequate interpretation and application of test results.

5 The test is best used for local application. Generalizations should be made with great caution.

6 Field observations should be made of results of application over a significant period of time.

7 Careful distinction should be made between fish mortality due to a physiological toxicant, and that due to lack of DO.

8 A uniform testing procedure is essential to effective action in water pollution control.

B Routine Procedure for Static Tests

1 Test organisms should be fish or other organisms of local significance.

- a The most sensitive species available should be selected, but:
- b They should be species which are amenable to captivity.
- c They should be accurately identified.
- d They should be relatively uniform in size. Individuals less than 3 inches in length are usually most convenient.
- e They should be healthy and thoroughly acclimated to the laboratory.
- f A careful record should be kept of their origin, handling, and condition.

2 Test water should preferably be taken from the receiving stream just above the discharge being evaluated or in a lake or estuary, beyond the influence of the discharge.

- a If this is unsuitable, cleaner but similar waters from a more remote station may be substituted.
- b Artificial "standard" waters are not recommended for general use, although many formulae have been proposed.
- c In estuarine situations, a series of tests (marine grid) should be run, using waters of high and low salinities as characteristic of the region.

3 Other experimental conditions

a **Temperature.** The tests should be performed at a uniform temperature in the upper part of the expected summer range, e.g., 25 ± 2°C for warm water fish, and 15 ± 2°C for cold water species.

b **Test containers** should be of glass, widemouthed "pickle jugs" or battery jars are satisfactory. Five and one gallon sizes are both useful, but the larger size is required for conclusive results.

c **Artificial aeration** should not be used to maintain the dissolved oxygen concentration. If this falls below approximately 4 or 5 ppm at any time during the test, fewer fish should be used per container or an auxiliary oxygenation procedure invoked that is designed to avoid undue loss of volatile toxicants.

d **The number of test animals** should not be less than 10 per concentration for reliable conclusions; these may be distributed between two or more containers.

e **Ratio of fish to solution.** There should be not more than one gram of fish per liter of test solution.

4 Experimental procedure

a **All dilutions** for a given run should be prepared **from the same sample**.

b **Duration.** Tests should be run for at least 48 hours, preferable 96.

c **Dead fish** should be removed as soon as observed. Survivors should be counted and recorded each 24 hours.

d **Feeding** during the test should be avoided.

e **Experimental concentrations.** Any appropriate series of concentrations may be used. A logarithmic series such as is suggested in Table I is very convenient.

TABLE I

A Guide to the Selection of Experimental Concentrations, Based on Progressive Bisection of Intervals on a Logarithmic Scale.

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
10.0	----	----	----	----
----	----	----	----	8.7
----	----	----	7.5	----
----	----	----	----	6.5
----	----	5.6	----	----
----	----	----	----	4.9
----	----	----	4.2	----
----	----	----	----	3.7
----	3.2	----	----	----
----	----	----	----	2.8
----	----	----	2.4	----
----	----	----	----	2.1
----	----	1.8	----	----
----	----	----	----	1.55
----	----	----	1.35	----
----	----	----	----	1.15
1.0	----	----	----	----

Effluents of unknown, mixed, or variable composition are usually best expressed as percent by volume; while pure substances, or specific analyzable components are usually expressed as milligrams per liter (ppm). A control or reference tank containing dilution water only (with no toxicant) is essential, to demonstrate that all experimental organisms would have survived had it not been for the toxicant being tested.

f **Expression of results.** The measure of relative toxicity is the tolerance limit (symbol: TL). The time of exposure "t" must be shown along with the percentage of fish surviving (written as a postscript). For example, a 96 hour TL50 (optional: $TL50^{96\ hr}$) of a toxic substance is that concentration in which 50% of the experimental organisms survive for 96 hours. (Figure 1)

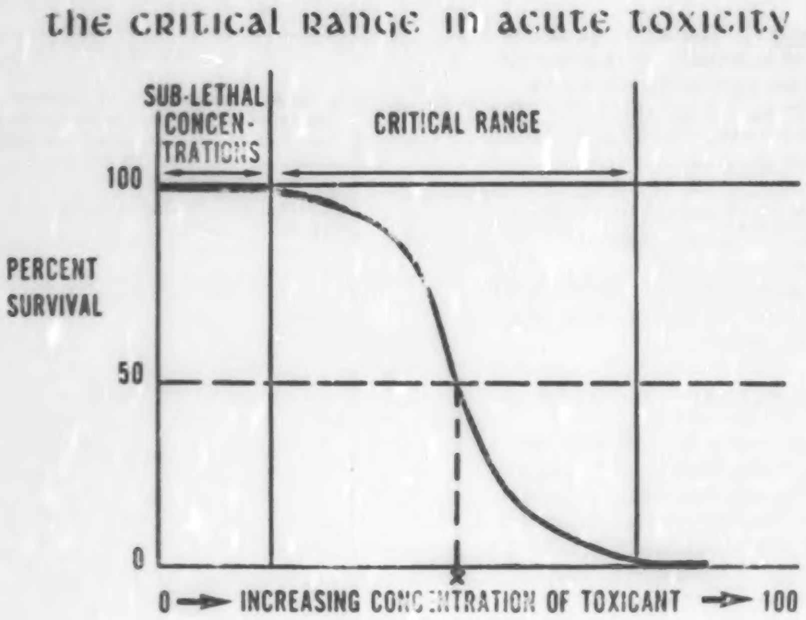


Figure 1
THE CRITICAL RANGE
X = TL50^t Concentration

1) A TL50^t is the equivalent of a median tolerance limit (TL$_m$^{t}).

2) This is analogous to the LC$_{50}$ (concentration survived by 50% of the population) of the toxicologist, but is more universally usable with the parameters encountered in the water environment, some of which (such as temperature) cannot be expressed as "concentrations."

3) TL50's for 96 hours or less are arbitrarily referred to as measures of "acute" toxicity, while TL50's for longer periods of time are variously referred to as sub-acute, chronic, etc.

C Special Problems of Static Tests

1 Unaerated aquaria with finite quantities of toxicant are not always satisfactory (static tests).

a The toxicant may be volatile.

b Toxic materials may be masked by a high BOD.

c The toxicant may be progressively adsorbed on container walk, fish slime, metabolized or otherwise changed so that actual concentrations in tanks change with time.

2 Standards or requirements other than those involving toxicity per se may be involved.

3 Preliminary and concurrent investigations

a Obtain all available information about unknown to be tested.

b Does the material lend itself to this type of test?

c Run feasible on the spot analyses including DO.

d Significant quantities of solutions removed from test containers for analysis should be replaced with similar volume of same dilution.

4 Wastes with a high BOD or COD

a Suggested preliminary tests:

1) Set up two identical exploratory tests.

2) Aerate one but not the other.

3) If great difference develops between them, special procedures are indicated.

b Oxygenation or aeration of dilution water before making dilutions may help.

c Renewal of solutions at stated intervals (12, 24, or 48 hours) is approved. Fish are not harmed by being carefully transferred from one container to another. It is useful where:

1) Initial DO is adequate but slowly exhausted.

2) Toxicant is volatile, progressively adsorbed, precipitated, or otherwise changed.

D Continuous Flow Procedures

1 Continuous flow procedures imply the continuous or periodic renewal of the solutions in the experimental containers, at the same time maintaining the stated concentrations (including control). The variety of devices and flow plans to accomplish this are almost infinite, two general principals will be outlined below: assaying and monitoring.

2 Continuous flow bioassay, general advantages (Figure 2)

a Materials with moderate oxygen demands may be tested.

b Materials which degrade or are volatile may be tested.

c Due to the constant removal of metabolic and other wastes, and the constant supply of fresh oxygenated water, fish may be fed and so maintained over a longer period of time. Containers must of course be maintained in reasonably clean condition.

E Test Concentrations and End Points for Continuous Flow Assays

1 Test concentrations are in general less restricted than for static tests. They need not be so high as to insure achieving the desired end point in 48 or 96 hours, although they may be so set if desired.

2 Geometric type series of concentrations are still desirable (See Table 1).

3 Sub-lethal levels may be tested over entire life histories of organisms to determine long range effects.

4 In general, the setup should be prepared, calibrated and operated for several days, or until the concentrations have become chemically and physically stabilized before introducing the fish or other experimental organisms.

F Total Fish Weight and Liquid Volume in Continuous Flow Assays

In general, the constant renewal of test solution might appear to make possible testing more or larger fish in less water. Actually, flow-through volume and total weight of fish must be so related that adequate oxygen is maintained. Furthermore over the longer periods of time involved, "lebensraum" (or territory) must be taken into account. Organisms must not be crowded to the extent that aggressive behavior and other ecological competitive factors are introduced.

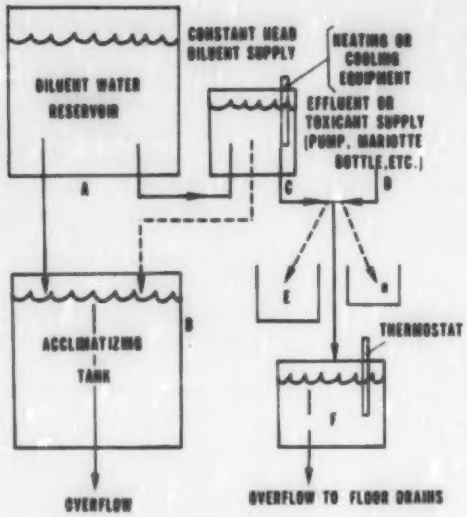

CONSTANT HEAD DILUENT SUPPLY
HEATING OR COOLING EQUIPMENT
DILUENT WATER RESERVOIR
EFFLUENT OR TOXICANT SUPPLY (PUMP, MARIOTTE BOTTLE, ETC.)
A
C
D
E
G
B
ACCLIMATIZING TANK
THERMOSTAT
F
OVERFLOW
OVERFLOW TO FLOOR DRAINS

Figure 1

BASIC SETUP FOR CONTINUOUS FLOW BIOASSAY

E, F, G represents one of several exposure tanks containing a graded series of dilutions of the toxicant, including one control with none.

G End Points or Reactions to be Evaluated by Continuous Flow Assays

1 The original and traditional end point of biological evaluations such as those discussed here was the death of the organism. This was simple, direct, and unequivocal. Current practice, however, often involves much more sophisticated reactions such as reduction in the reproductive capacity, or a change in the breathing rate (movement of gills).

H Special Problems of Flow Through Bioassays

1 Due to the physical requirements of maintaining stated concentrations of chemicals over long periods of time, laboratory setups are usually complicated and always require attention and maintenance.

2 The problem of disease control frequently develops in populations held over a long period of time.

3 Water and/or power failure may jeopardize an assay experiment after months of time have been expended.

4 The expense of a long continued test may not be justified by the result.

5 The above points demonstrate that in general flow through bioassays are not adapted to day-to-day routine toxicity determinations.

III REPORTING INTERPRETATION AND APPLICATION OF BIOASSAY RESULTS

A Reporting

1 Reports should include an orderly tabulation of all pertinent data such as:

a The type of setup used and duration of test

b Identity of experimental animals

c Their source, history, average size and condition, and number used per concentration

d Source of, and chemical and physical analysis of experimental dilution water

e Experimental temperature

f Volumes of experimental liquid in each container

g Records of routine analyses such as DO and pH

h Records of chemical analyses of toxicants in experimental tanks

i TL50^t or other end point, and data from which it was determined.

B Interpretation and Application

1 The TL50^t is an estimate of the mid-point of the critical concentration range the interval between the highest concentration at which all test animals survive, and the lowest at which they all die (Figure 1).

2 The final step is to extrapolate from this well established mid concentration to a safe concentration well below the "critical concentration range." Extrapolating or rather: "application factors" to accomplish this are still under development and will probably not be fully developed for many years. Available data indicate that these factors must be variable according to the toxicant in question acting in combination with the receiving water in question, and considering the entire aquatic community.

3 For a more complete discussion, see FWPCA Water Quality Criteria (Reference No. 9) pp. 55-72.

4 Other considerations

a Radioactive wastes must be evaluated with regard to their chemical toxicity as well as their radioactivity.

b Sub-acute levels of many toxicants such as lead, arsenic, cadmium, etc. may exert a low level chronic toxicity over a long period of time.

c "Safe levels" of a waste in regard to toxicity to aquatic life may still exceed standards of other types such as color, organic content, suspended solids, etc.

IV BIOMONITORING AS COMPARED TO BIOASSAY (Figure 3)

A Bioassay is (as stated above) the evaluation of the effects of stated concentrations of the test material for given periods of time.

B Biomonitoring is the use of organisms to detect change in an effluent (surveillance). It operates continuously and indefinitely.

C Bioassays typically involve relatively small flows and employ often especially prepared (perhaps repeatedly prepared) batches of experimental material, while biomonitoring typically involves larger flows, from operating industries or other installations.

D Bioassays basically determine:

1 Is the substance deleterious, and if so:

2 How deleterious is it?

E Biomonitoring is useful to

1 Demonstrate the continuous suitability of an effluent (or a predetermined dilution thereof) for the survival of the test organism.

2 Detect a change (usually deleterious) in the biological acceptability of the effluent.

3 To detect a change in the effect of a mixture of the effluent and the receiving water on the test organism (i.e. to detect a change in the receiving water).

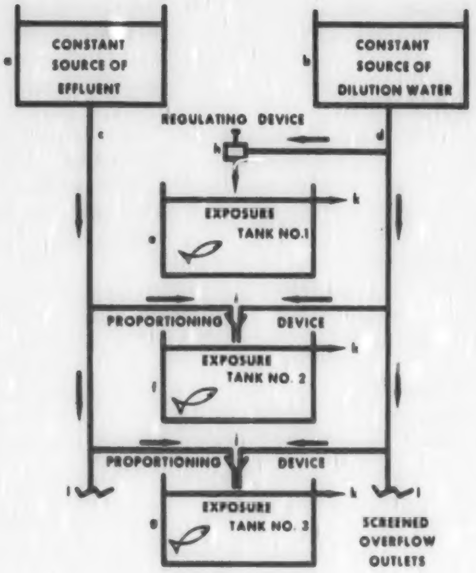

a
CONSTANT SOURCE OF EFFLUENT
b
CONSTANT SOURCE OF DILUTION WATER
REGULATING DEVICE
c
d
h
EXPOSURE TANK NO.1
e
k
PROPORTIONING DEVICE
i
EXPOSURE TANK NO. 2
f
k
PROPORTIONING DEVICE
i
j
EXPOSURE TANK NO. 3
g
k
j
SCREENED OVERFLOW OUTLETS

Figure 3
BASIC SETUP FOR BIOMONITORING

F Biomonitoring was originally effective only with relatively fast acting materials, or in situations where large changes might occur rather quickly (as for example, the accidental (?) dumping of a vat of waste pickle liquor). Recent developments in the field of biotelemetry now make it feasible to "wire" a fish with electrodes (like the astronauts) and so to immediately record electronically any sudden or subtle change in the effluent which affects the physiological parameters being monitored on the live fish.

REFERENCES

1 American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 12th Ed. (13th edition when available). New York. 1965. (13th edition: 1970).

2 Doudoroff, P., et al. Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish. Sew. and Ind. Wastes, Vol. 23, No. 11. November 1951.

3 Doudoroff, P. and Katz, M. Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish. I Alkalies, Acids and Inorganic Gases, Sew. and Ind. Wastes, Vol. 22, No. 11, p. 1432. November 1950.

4 Ellis, M.M., Westfall, B.A. and Ellis, M.D. Determination of Water Quality. Research Report 9, U.S. Fish and Wildlife Service, 122 pp. 1946.

5 Hart, W.B., Doudoroff, P. and Greenbank, J. The Evaluation of the Toxicity of Industrial Wastes, Chemicals and Other Substances to Fresh-Water Fishes. The Atlantic Refining Company, Philadelphia, Pa. 317 pp.

6 Hart, W.B., Weston, R.F. and DeMann, J.F. An Apparatus for Oxygenating Test Solutions in Which Fish are Used as Test Animals for Evaluating Toxicity. Trans. Am. Fisheries Soc. 75:228. 1948.

7 Jackson, H.W. and Brungs, W.A. Biomonitoring of Industrial Effluents. Proc. 21st Ind. Waste Conf. Purdue Univ. (Eng. Extension Bull. No. 121). p. 117. 1966.

8 Mount, D.I. and Brungs, W.A. A Simplified Dosing Apparatus for Fish Toxicology Studies. Water Research 1:21. 1967.

9 U.S. Department of the Interior, FWPCA, Report of the Committee on Water Quality Criteria. USGPO, Washington, DC. April 1, 1968.

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

USING BENTHIC BIOTA IN WATER QUALITY EVALUATIONS

I BENTHOS ARE ORGANISMS GROWING ON OR ASSOCIATED PRINCIPALLY WITH THE BOTTOM OF WATERWAYS

Benthos is the noun.

Benthonic, benthal and benthic are adjectives.

II THE BENTHIC COMMUNITY

A Composed of a wide variety of life forms that are related because they occupy "common ground"--the waterways bottom substrates. Usually they are attached or have relatively weak powers of locomotion. These life forms are:

1 Bacteria

A wide variety of decomposers work on organic materials, breaking them down to elemental or simple compounds (heterotrophic). Other forms grow on basic nutrient compounds or form more complex chemical compounds (autotrophic).

2 Algae

Single-cell plants that are the basic producers of food that nurtures the animal components of the community.

3 Flowering Aquatic Plants (Pondweeds)

The largest flora, composed of complex and differentiated tissues. Many are rooted.

4 Micro-Fauna

Animals that pass through a U.S. Standard Series No. 30 sieve, but are retained on a No. 100 sieve. Examples are rotifers and microcrustaceans. Some forms have organs for attachment to substrates, while others burrow into soft materials or occupy the interstices between rocks, floral or faunal materials.

5 Macro-Fauna

Animals that are retained on a No. 30 sieve. This group includes the insects, worms, molluscs, and occasionally fish. Fish are not normally considered as benthos, though there are bottom dwellers such as sculpins and darters.

B It is a self-contained community, though there is interchange with other communities. For example: Plankton settles to it, fish prey on it and lay their eggs there, terrestrial detritus is added to it, and many aquatic insects migrate from it to the terrestrial environment for their mating cycles.

C It is a stationary water quality monitor. The low motility of the biotic components requires that they "live with" the quality changes of the over-passing waters. Changes imposed in the long-lived components remain visible for extended periods, even after the cause has been eliminated. Only time will allow a cure for the community by migration and reproduction.

III HISTORY OF BENTHIC OBSERVATIONS

A Ancient literature records the vermin associated with fouled waters.

B 500-year-old fishing literature refers to animal forms that are fish food and used as bait.

C The scientific literature associating biota to water pollution problems is over 100 years old (Mackenthun and Ingram, 1964).

D Early this century, applied biological investigations were initiated.

1 The entrance of State boards of Health into water pollution control activities.

2 Creation of state conservation agencies.

3 Industrialization and urbanization.

4 Growth of limnological programs at universities.

E A decided increase in benthic studies occurred in the 1950 decade, and much of today's activities are strongly influenced by developmental work conducted during this period. Some of the reasons for this are:

1 Movement of the universities from "academic biology" to applied pollution programs.

2 Entrance of the federal government into enforcement aspects of water pollution control.

3 A rising economy and the development of federal grant systems.

4 Environmental Protection Programs are a current stimulus.

IV WHY THE BENTHOS?

A It is a natural monitor

B The community contains all of the components of an ecosystem.

1 Reducers

2 Producers

3 Consumers

a Herbivores

b Predators

C Economy of Survey

1 Manpower

2 Time

3 Equipment

D Extensive Supporting Literature

V REACTIONS OF THE COMMUNITY TO POLLUTANTS

A Destruction of Organism Types

1 Beginning with the most sensitive forms, pollutants kill in order of sensitivity until the most tolerant form is the last survivor. This results in a reduction of variety or diversity of organisms.

2 The usual order of macroinvertebrate disappearance on a sensitivity scale below pollution sources is shown in Figure 2.

Stoneflies
Mayflies
Caddisflies
Amphipods
Isopods
Midges
Oligochaetes

As water quality improves, these reappear in the same order.

B The Number of Survivors Increase

1 Competition and predation are reduced between forms.

2 When the pollutant is a food (plants, fertilizers, animals, organic materials).

C The Number of Survivors Decrease

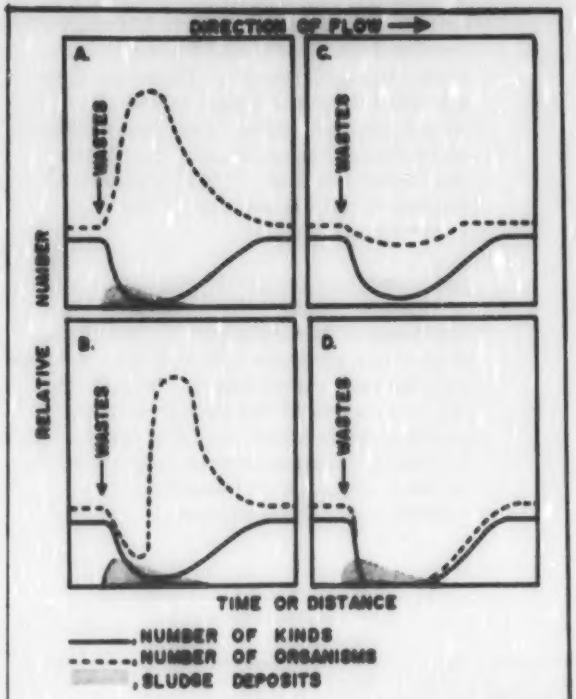
1 The material added is toxic or has no food value.

2 The material added produces toxic conditions as a byproduct of decomposition (e.g., large organic loadings produce an anaerobic environment resulting in the production of toxic sulfides, methanes, etc.)

D The Effects May be Manifest in Combinations

1 Of pollutants and their effects.

2 Vary with longitudinal distribution in a stream. (Figure 1)



Four basic responses of bottom animals to pollution. A. Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms. B. Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of byproducts of organic decomposition inhibit the tolerant forms; in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food. C. Toxic materials eliminate the sensitive bottom animals; sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms. Very toxic materials may eliminate all organisms below a waste source. D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms. Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth.

Figure 1

E Tolerance Grouping (Figure 2)

Flexibility must be maintained in the establishment of tolerance lists based on the response of organisms to the environment because of complex relationships among varying environmental conditions. Some general tolerance patterns can be established. Stonefly nymphs, mayfly naiads, hellgrammites, and caddisfly larvae represent a grouping (sensitive or intolerant) that is quite sensitive to environmental changes. Blackfly larvae, scuds, sowbugs, snails, fingernail clams, dragonfly nymphs, damselfly nymphs, and most kinds of midge larvae are intermediate (facultative or intermediate) in tolerance. Sludgeworms, some kinds of midge larvae (bloodworms), and some leeches are tolerant to comparatively heavy loads of organic pollutants. Sewage mosquitoes and rat-tailed maggots are tolerant of anaerobic environments.

F Structural Limitations

The morphological structure of a species limits the type of environment it may occupy. Species with complex appendages and exposed, complicated respiratory structures, such as stonefly nymphs, mayfly numphs, and caddisfly larvae, that are subjected to a constant deluge of settleable particulate matter soon abandon the polluted area because of the constant preening required to maintain mobility or respiratory functions; otherwise, they are soon smothered.

Species without complicated external structures, such as bloodworms and sludgeworms, are not so limited in adaptability. A sludgeworm, for example, can burrow in a deluge of particulate organic matter and flourish on the abundance of "manna." Morphology also determines the species that are found in riffles, on vegetation, on the bottom of pools, or in bottom deposits.

VI SAMPLING PROCEDURES

A Fauna

1 Qualitative sampling determines the variety of species occupying an area. Samples may be taken by any method that will capture representatives of the species present. Collections from such samplings indicate changes in the environment, but generally do not accurately reflect the degree of change. Mayflies, for example, may be reduced from 100 to 1 per square foot. Qualitative data would indicate the presence of both species, but might not necessarily delineate the change in predominance from mayflies to sludgeworms.

2 Quantitative sampling is performed to observe changes in predominance. The most common quantitative sampling tools are the Petersen and Ekman dredges and the Surber stream bottom or square-foot sampler. Of these, the Petersen dredge samples the widest variety of substrates. The Ekman dredge is limited to fine-textured and soft substrates, such as silt and sludge. The Surber sampler is designed for sampling riffle areas; it requires moving water to transport dislodged organisms into its net and is limited to depths of two feet or less.

3 The collected sample is screened with a standard sieve to concentrate the organisms; these are sorted from the retained material, and the number of each kind determined. Data are then adjusted to number per unit area, usually to the number per square foot of bottom or occasionally to number per square meter. This adjustment standardized the method of data expression.

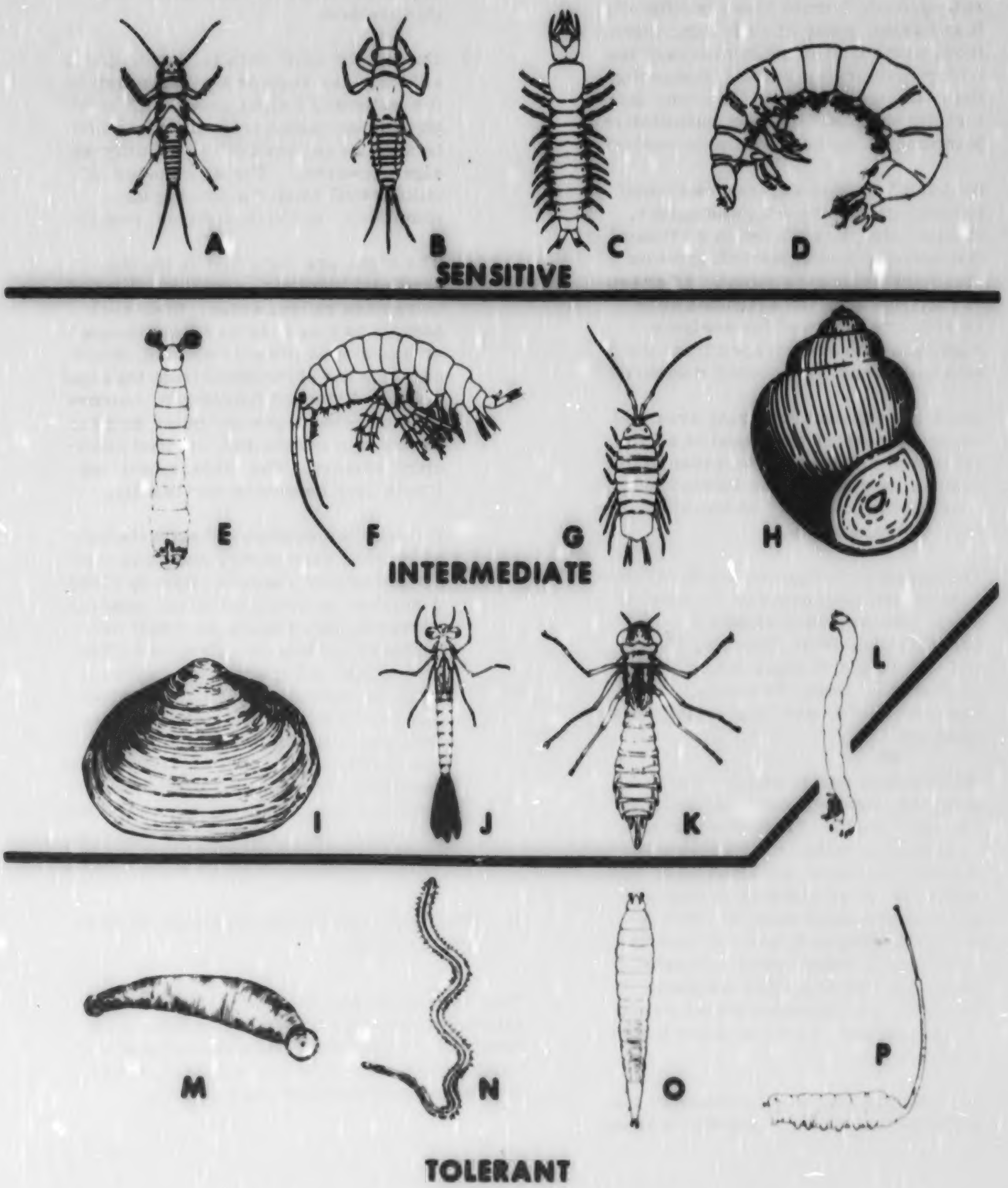
4 Independently, neither qualitative nor quantitative data suffice for thorough analyses of environmental conditions. A cursory examination to detect damage may be made with either method, but a combination of the two gives a more precise determination. If a choice must be made, quantitative sampling would be best, because it incorporates a partial qualitative sample.

REPRESENTATIVE BOTTOM-DWELLING MACROANIMALS

Drawings from Geckler, J., K. M. Mackenthun and W. M. Ingram, 1963. Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control, DHEW, PHS, Cincinnati, Ohio, Pub. No. 999-WP-2.

A	Stonefly nymph	(Plecoptera)	I	Fingernail clam	(Sphaeriidae)
B	Mayfly nymph	(Ephemeroptera)	J	Damselfly nymph	(Zygoptera)
C	Hellgrammite or Dobsonfly larvae	(Megaloptera)	K	Dragonfly nymph	(Anisoptera)
D	Caddisfly larvae	(Trichoptera)	L	Bloodworm or midge fly larvae	(Chironomidae)
E	Black fly larvae	(Simuliidae)	M	Leech	(Hirudinea)
F	Scud	(Amphipoda)	N	Sludgeworm	(Tubificidae)
G	Aquatic sowbug	(Isopoda)	O	Sewage fly larvae	(Psychodidae)
H	Snail	(Gastropoda)	P	Rat-tailed maggot	(Tubifera-Eristalis)

KEY TO FIGURE 2


A
B
C
D
SENSITIVE
E
F
G
H
INTERMEDIATE
I
J
K
L
M
N
O
P
TOLERANT

B Flora

1 Direct quantitative sampling of naturally growing bottom algae is difficult. It is basically one of collecting algae from a standard or uniform area of the bottom substrates without disturbing the delicate growths and thereby distort the sample. Indirect quantitative sampling is the best available method.

Artificial substrates, such as wood blocks, glass or plexiglass slides, bricks, etc., are placed in a stream. Bottom-attached algae will grow on these artificial substrates. After two or more weeks, the artificial substrates are removed for analysis. Algal growths are scraped from the substrates and the quantity measured.

Since the exposed substrate area and exposure periods are equal at all of the sampling sites, differences in the quantity of algae can be related to changes in the quality of water flowing over the substrates.

2 The quantity of algae on artificial substrates can be measured in several ways. Microscopic counts of algal cells (Cooke, 1956; Gumtow, 1955) and dry weight of algal material (Castenholz, 1960; Grezenda and Brehmer, 1960) are long established methods.

Microscopic counts involve thorough scraping, mixing, and suspension of the algal cells. From this mixture an aliquot of cells is withdrawn for enumeration under a microscope. Dry weight is determined by drying and weighing the algal sample, then igniting the sample to burn off the algal materials, leaving inert inorganic materials that are again weighed. The difference between initial weight and weight after ignition is attributed to algae.

Any organic sediments, however, that settle on the artificial substrate along with the algae are processed also. Thus, if organic wastes are present appreciable errors may enter into this method.

3 During the past decade, chlorophyll analysis has become a popular method for estimating algal growth. Chlorophyll is extracted from the algae and is used as an index of the quantity of algae present. The advantages of chlorophyll analysis are rapidity, simplicity, and vivid pictorial results.

The algae are scrubbed from the artificial substrate samples, filtered to remove excess water; then each sample is placed (with filter) in equal volumes of acetone or alcohol, which extracts the chlorophyll from the algal cells. A second filtering to remove the extracted algae and other detritus produces a non-turbid, colored chlorophyl extract. The chlorophyll extracts may be compared visually.

Because the chlorophyll extracts fade with time, colorimetry should be used for permanent records. Harvey (1934) describes an artificial color standard. Richards with Thompson (1952) describes a method of colorimetry that determines the quantities of various kinds of chlorophyll along with other plant and animal pigments. For routine records, simple colorimeters will suffice. At very high chlorophyll densities, interference with colorimetry occurs, which must be corrected through serial dilution of the sample or with a nomograph.

VII FACTORS INVOLVED IN DATA INTERPRETATION

Two very important factors in data evaluation are a thorough knowledge of conditions under which the data were collected and a critical assessment of the reliability of the data's representation of the situation.

A Maximum-Minimum Values

The evaluation of physical and chemical data to determine their effects on aquatic organisms is primarily dependent on maximum and minimum observed values. The mean is useful only when the data are relatively uniform. The minimum or maximum values usually create acute conditions in the environment.

B Identification

Precise identification of organisms to species requires a specialist in limited taxonomic groups. Many immature aquatic forms have not been associated with the adult species. Therefore, one who is certain of the genus but not the species should utilize the generic name, not a potentially incorrect species name. The method of interpreting biological data on the basis of numbers of kinds and numbers of organisms will typically suffice.

C Lake and Stream Influence

Physical characteristics of a body of water also affect animal populations. Lakes or impounded bodies of water support different faunal associations from rivers. The number of kinds present in a lake may be less than that found in a stream because of a more uniform habitat. A lake is all pool, but a river is composed of both pools and riffles. The nonflowing water of a lake exhibits a more complete settling of particulate organic matter that naturally supports a higher population of detritus consumers. For these reasons, the bottom fauna of a lake or impoundment cannot be directly compared with that of a flowing stream.

D Extrapolation

How can bottom-dwelling macrofauna data be extrapolated to other environmental components? It must be borne in mind that a component of the total environment is being sampled. If the sampled component exhibits changes, then so must the other interdependent components of the environment. For example, a clean stream with a wide variety of desirable bottom organisms would be expected to have a wide variety of desirable bottom fishes; when pollution reduces the number of bottom organisms, a comparable reduction would be expected in the number of fishes. Moreover, it would be logical to conclude that any factor that eliminates all bottom organisms would eliminate most other aquatic forms of life.

VIII IMPORTANT ASSOCIATED ANALYSES

A The Chemical Environment

1 Dissolved oxygen

2 Nutrients

3 Toxic materials

4 Acidity and alkalinity

5 Etc.

B The Physical Environment

1 Suspended solids

2 Temperature

3 Light penetration

4 Sediment composition

5 Etc.

IX AREAS IN WHICH BENTHIC STUDIES CAN BEST BE APPLIED

A Damage Assessment

If a stream is suffering from pollutants, the biota will so indicate. A biologist can determine damages by looking at the "critter" assemblage in a matter of hours. Usually, if damages are not found, it will not be necessary to alert the remainder of the agency's staff, pack all the equipment, pay travel and per diem, and then wait five days before enough data can be assembled to begin evaluation.

B By determining what damages have been done, the potential cause "list" can be reduced to a few items for emphasis and the entire "wonderful worlds" of science and engineering need not be practiced with the result that much data are discarded later because they were not applicable to the problem being investigated.

C Good benthic data coupled with chemical, physical and engineering materials can be used to predict the direction of future changes and to estimate the amount of pollutants that need to be removed from the waterways.

REFERENCES

1 Castenholz, R.W. Season Changes in the Attached Algae of Freshwater and Saline Lakes in the Lower Grand Coulee, Washington. Limnology and Oceanography 5 (1):1. 1960.

2 Cooke, W.B. Continuous Sampling of Trickling Filter Populations I. Procedures. Sewage and Industrial Wastes 30(1):21. 1958.

3 Grezenda, A.R. and Brehmer, M.L. A Quantitative Method for Collection and Measurement of Stream Periphyton. Limnology and Oceanography 5(2):190. 1960.

4 Gumtow, R.B. An Investigation of the Periphyton in a Riffle of the West Gallatin River, Montana. Trans. Am. Microscopical Society 74(3):278. 1955.

5 Harvey, H.W. Measurement of Phytoplankton Populations. Journal Marine Biological Assoc. 19:761. 1934.

6 Hynes, H.B.N. The Ecology of Running Waters. Univ. Toronto Press. 1970.

7 Keup, L.E., Ingram, W.M. and Mackenthun, K.M. The Role of Bottom Dwelling Macrofauna in Water Pollution Investigations. USPHS Environmental Health Series Publ. No. 999-WP-38, 23 pp. 1966.

8 Keup, L.E., Ingram, W.M. and Mackenthun, K.M. Biology of Water Population: A Collection of Selected Papers on Stream Pollution, Waste Water, and Water Treatment. Federal Water Pollution Control Administration Pub. No. CWA-3, 290 pp. 1967.

9 Mackenthun, K.M. The Practice of Water Pollution Biology. FWQA. 281 pp. 1969.

10 Richards, F.A. and Thompson, T.G. The Estimation and Characterization of Plankton Populations by Pigment Analysis II. A Spectrophotometric Method for the Estimation of Plankton Pigments. Journal Marine Research 11(2):156. 1952.

11 Stewart, R.K., Ingram, W.M. and Mackenthun, K.M. Water Pollution Control, Waste Treatment and Water Treatment: Selected Biological References on Fresh and Marine Waters. FWPCA Pub. No. WP-23, 126 pp. 1966.

This outline was prepared by Lowell E. Keup, Chief, Technical Studies Branch, Div. of Technical Support, EPA, Washington, D.C. 20242.

ATTACHED GROWTHS
(Periphyton or Aufwuchs)

I The community of attached microscopic plants and animals is frequently investigated during water quality studies. The attached growth community (periphyton) and suspended growth community (plankton) are the principal primary producers in waterways--they convert nutrients to organic living materials and store light originating energy through the processes of photosynthesis. In extensive deep waters, plankton is probably the predominant primary producer. In shallow lakes, ponds, and rivers, periphyton is the predominant primary producer. During the past two decades, investigators of microscopic organisms have increasingly placed emphasis on periphytic growths because of inherent advantages over the plankton when interpreting data from surveys on flowing waters:

A Blum (1956) "....workers are generally agreed that no distinctive association of phytoplankton is found in streams, although there is some evidence of this for individual zooplankters (animals) and for a few individual algae and bacteria. Plankton organisms are often introduced into the current from impoundments, backwater areas or stagnant arms of the stream.... Rivers whose plankton is not dominated by species from upstream lakes or ponds are likely to exhibit a majority of forms which have been derived from the stream bottom directly and which are thus merely facultative or opportunistic plankters. "

B "The transitory nature of stream plankton makes it nearly impossible to ascertain at which point upstream agents producing changes in the algal population were introduced, and whether the changes occurred at the sampling site or at some unknown point upstream. In contrast, bottom algae (periphyton) are true components of the stream biota. Their sessile-attached mode of life subjects them to the quality of water continuously flowing over them. By observing the longitudinal distribution of bottom algae within a stream, the sources of the agents producing the change can be traced (back-tracked)" (Keup, 1966).

II TERMINOLOGY

A Two terms are equally valid and commonly in use to describe the attached community of organisms. Periphyton literally means "around plants" such as the growths overgrowing pond-weeds; through usage this term means the attached film of growths that rely on substrates as a "place-to-grow" within a waterway. The components of this growth assemblage consists of plants, animals, bacteria, etc. Aufwuchs is an equally acceptable term [probably originally proposed by Seligo (1905)]. Aufwuchs is a German noun without equivalent english translation; it is essentially a collective term equivalent to the above American (Latin root) term - Periphyton. (For convenience, only, PERIPHYTON, with its liberal modern meaning will be used in this outline.)

B Other terms, some rarely encountered in the literature, that are essentially synonymous with periphyton or describe important and dominant components of the periphytic community are: Nereiden, Bewuchs, Laison, Belag, Besatz, attached, sessile, sessile-attached, sedentary, seeded-on, attached materials, slimes, slime-growths, and coatings.

The academic community occasionally employs terminology based on the nature of the substrates the periphyton grows on (Table 1).

TABLE 1

Periphyton Terminology Based on Substrate Occupied

Substrate	Adjective
various	epiholitic, nereiditic, sessile
plants	epiphytic
animals	epizooic
wood	epidendritic, epixylonic
rock	epilithic

[After Srameck-Husek (1946) and via Sladeckova (1962)] Most above listed latin-root adjectives are derivatives of nouns such as epihola, epiphyton, epizoa, etc.

III Periphyton, as with all other components of the environment, can be sampled qualitatively (what is present) and quantitatively (how much or many are present).

A Qualitative sampling can be performed by many methods and may extend from direct examination of the growths attached to a substrate to unique "cuttings" or scrapings. It may also be a portion of quantitative sampling.

B Quantitative sampling is difficult because it is nearly impossible to remove the entire community from a standardized or unit area of substrate.

1 Areas scraped cannot be determined precisely enough when the areas are amorphous plants, rocks or logs that serve as the principal periphyton substrates.

2 Collection of the entire community within a standard area usually destroys individual specimens thereby making identification difficult (careful scraping can provide sufficient intact individuals of the species present to make qualitative determinations); or the process of collection adds sufficient foreign materials (i.e. detritus, substrate, etc.) so that some commonly employed quantitative procedures are not applicable.

IV Artificial substrates are a technique designed to overcome the problems of direct sampling. They serve their purpose, but cannot be used without discretion. They are objects standardized as to surface area, texture, position, etc. that are placed in the waterway for pre-selected time periods during which periphytic growths accumulate. They are usually made of inert materials, glass being most common with plastics second in frequency. Over fifty various devices and methods of support or suspension of the substrates have been devised (Sladeckova, 1962) (Weber, 1966) (Thomas, 1968).

V ARTIFICIAL SUBSTRATE PLACEMENT

A Position or Orientation

1 Horizontal - Includes effects of settled materials.

2 Vertical - Eliminates many effects of settled materials.

B Depth (light) - A substrate placed in lighted waters may not reflect conditions in a waterway if much of the natural substrate (bottom) does not receive light or receives light at reduced intensity. (Both excessive light and a shortage of light can inhibit growths and influence the kinds of organisms present.)

C Current is Important

1 It can prevent the settling of smothering materials.

2 It flushes metabolic wastes away and introduces nutrients to the colony.

VI THE LENGTH OF TIME THE SUBSTRATE IS EXPOSED IS IMPORTANT.

A The growths need time to colonize and develop on the recently introduced substrate.

B Established growths may intermittently break-away from the substrate because of current or weight induced stresses, or "over-growth" may "choke" the attachment layers (nutrient, light, etc. restrictions) which then weaken or die allowing release of the mass.

C A minimum of about ten days is required to produce sufficient growths on an artificial substrate; exposures exceeding a longer time than 4-6 weeks may produce "erratic results" because of sloughing or the accumulation of senile growths in situations where the substrate is artificially protected from predation and other environmental stresses.

VII Determining the variety of growths present is presently only practical with microscopic examination. (A few micro-chemical procedures for differentiation show promise--but, are only in the early stages of development.)

VIII DETERMINING THE QUANTITY OF GROWTH(S)

A Direct enumeration of the growths while attached to the substrate can be used, but is restricted to the larger organisms because (1) the problem of maintaining material in an acceptable condition under the short working distances of the objective lenses on compound microscopes, and (2) transmitted light is not adequate because of either opaque substrates and/or the density of the colonial growths.

B Most frequently, periphyton is scraped from the substrate and then processed according to several available procedures, the selection being based on the need, and use of the data.

1 Aliquots of the sample may be counted using methods frequently employed in plankton analysis.

a Number of organisms

b Standardized units

c Volumetric units

d Others

2 Gravimetric

a Total dry weight of scrapings

b Ash-free dry weight (eliminates inorganic sediment)

c A comparison of total and ash-free dry weights

3 Volumetric, involving centrifugation of the scrapings to determine a packed biomass volume.

4 Nutrient analyses serve as indices of the biomass by measuring the quantity of nutrient incorporated.

a Carbon

1) Total organic carbon

2) Carbon equivalents (COD)

b Organic nitrogen

c Phosphorus - Has limitations because cells can store excess above immediate needs.

d Other

5 Chlorophyll and other bio-pigment extractions.

6 Carbon-14 uptake

7 Oxygen production, or respiratory oxygen demand

IX EXPRESSION OF RESULTS

A Qualitative

1 Forms found

2 Ratios of number per group found

3 Frequency distribution of varieties found

4 Autotrophic index (Weber)

5 Pigment diversity index (Odum)

B Quantitative

1 Areal basis--quantity per square inch, foot, centimeter, or meter. For example:

a 16 mgs/sq. inch

b 16,000 cells/sq. inch

2 Rate basis. For example:

a 2 mgs/day, of biomass accumulation

b 1 mg O_2/mg of growth/hour

REFERENCES

1 Blum, J.L. The Ecology of River Algae. Botanical Review. 22:5:291. 1956.

2 Dumont, H.J. A Quantitative Method for the Study of Periphyton. Limnol. Oceanogr. 14(2):584-595.

3 Keup, L.E. Stream Biology for Assessing Sewage Treatment Plant Efficiency. Water and Sewage Works. 113:11-411. 1966.

4 Seligo, A. Uber den Ursprung der Fischnahrung. Mitt. d. Westpr. Fisch. -V. 17:4:52. 1905.

5 Sladeckova, A. Limnological Investigation Methods for the Periphyton Community. Botanical Review. 28:2:286. 1962.

6 Srameck-Husek. (On the Uniform Classification of Animal and Plant Communities in our Waters). Sbornik MAP 20:3:213. Orig. in Czech. 1946.

7 Thomas, N.A. Method for Slide Attachment in Periphyton Studies. Manuscript. 1968.

8 Weber, C.I. Methods of Collection and Analysis of Plankton and Periphyton Samples in the Water Pollution Surveillance System. Water Pollution Surveillance System Applications and Development Report No. 19, FWPCA, Cincinnati. 19 +pp. (multilith). 1966.

9 Weber, C.I. Annual Bibliography Midwest Benthological Society. Periphyton. 1014 Broadway, Cincinnati, OH 45202.

10 Hynes, H.B.N. The Ecology of Running Waters. Univ. Toronto Press. 555 pp. 1970.

This outline was prepared by Lowell E. Keup, Chief, Technical Studies Branch, Division of Technical Support, EPA, Washington, DC 20242.

STREAM INVERTEBRATE DRIFT

I Invertebrates which are part of the benthos, but under certain conditions become carried downstream in appreciable numbers, are known as drift.

A Groups which have members forming a conspicuous part of the drift include the insect orders Ephemeroptera, Trichoptera, Plecoptera and the crustacean order Amphipoda.

B Other invertebrate groups exhibit drift patterns.

II THREE BASIC TYPES OF DRIFT ARE RECOGNIZED

A Catastrophic Drift

Floods wash numerous benthic organisms downstream. Application of pesticides may also cause such drift.

B Constant Drift (Incidental or Adventitious)

Organisms are constantly being dislodged from the substrate during normal activities and carried downstream.

C Periodic (Diel) or Behavioral Drift

In contrast to the other categories, this is a specific behavior pattern and related to circadian activity rhythms.

1 Seasonal drift occurs, for example, in some maturing stoneflies which drift downstream for emergence. This is another reason for a serious consideration of drift in bottom fauna sampling since such presence of stoneflies could easily be misinterpreted.

2 Periodic or diel drift occurs in peaks for successive 24-hour periods.

a Night-active. Light intensity is the phase-setting mechanism.

b Day-active. Water temperature appears to be the phase-setter.

III DIEL DRIFT

A Diel activity rhythms generally include two peaks during the 24-hour period; one major and the other minor.

1 The bigeminus type in which the major peak occurs first (after sunset).

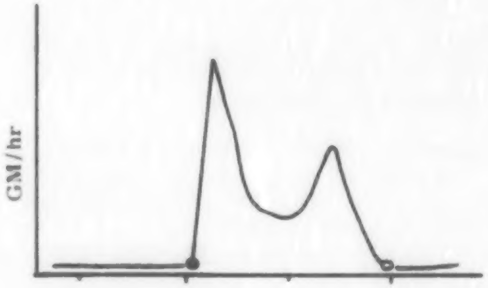


FIGURE 1

2 The alternans pattern with the major peak occurring last.

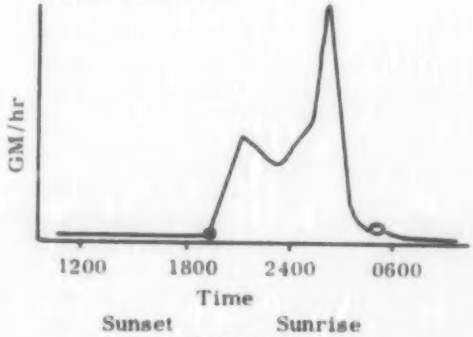


FIGURE 2

B Drift Rate and Density (Waters, 1969)

1 Drift rate defined is "...the quantity of organisms passing a width transect or portion thereof, per unit time;

it is a measure of displacement or the movement of organisms from one place to another."

2 Drift density "...is the quantity of organisms per unit volume of water, in much the same way as plankton density can be defined."

IV IMPLICATIONS FOR BIOLOGICAL SAMPLING

A The drift from productive upstream reaches may support a fish population existing in relatively barren stream sections.

B Drift will colonize artificial substrates, such as suspended rock baskets, when placed in such habitats.

C A bottom sampler such as the Surber, could also be sampling drift when only resident benthic organisms are intended to be collected. This would depend on the hour of collection and length of time the Surber sampler is in the water.

D Application of drift studies have been widely used in pesticide related studies. In conjunction with such studies, Dimond concluded that:

1 The status of drift is a much better indicator of the steady state and of total productivity than is the status of the bottom fauna.

2 Bottom sampling, however, is superior when analyzing survival and recovery of the quality of population.

3 A combination of both in such a sampling program would be most likely to yield the most useful data.

E Drift sampling techniques have been useful for recovery of large numbers of sand-dwelling mayflies, which were once rarely collected.

V MAJOR TAXA INVOLVED IN DRIFT

A The crustacean order Amphipoda

1 Gammarus species

2 Hyalella azteca

B The Insect Orders

1 Ephemeroptera

Baetis species (apparently universal)

2 Plecoptera

3 Trichoptera

4 Diptera
Simuliidae

5 Elmidae

C The main groups exhibiting very high drift rates include: Baetis, some Gammarus species, and some Simuliidae.

REFERENCES

1 Anderson, N.H. Biology and Downstream Drift of some Oregon Trichoptera. Can. Entom. 99:507-521. 1967.

2 Dimond, John B. Pesticides and Stream Insects. Bull. 23, Maine Forest Service, 21 pp. 1967.

3 Dimond, John B. Evidence that drift of Stream Benthos is Density Related. Ecology 48:855-857. 1967.

4 Pearson, William D., and Kramer, Robert H. A Drift Sampler driven by a Waterwheel. Limnology and Oceanography 14(3):462-465.

5 Reed, Roger J. Some Effects of DDT on the Ecology of Salmon Streams in Southeastern Alaska. Spec. Sci. Report-Fisheries 542:1-15. U.S. Bureau Comm. Fisheries. 1966.

6 Waters, Thomas F. Interpretation of Invertebrate Drift in Streams. Ecology 46 (3):327-334. 1965.

7 Waters, Thomas F. Diurnal Periodicity in the Drift of a Day-active Stream Invertebrate. Ecology 49:152. 1968.

8 Waters, Thomas F. Invertebrate Drift-Ecology and Significance to Stream Fishes. (T.G. Northco e, Ed.) Symposium Salmon and Trout in Streams. University of British Columbia, Vancouver. pp. 121-134. 1969.

This outline was prepared by R. M. Sinclair, Aquatic Biologist, National Training Center, Office of Water Programs, EPA, Cincinnati, OH 45268.

SECTION D

ANALYSIS AND USE OF BIOLOGICAL DATA

	Outline Number
The Interpretation of Biological Data with Reference to Water Quality	D- 1
Significance and Interpretation of Biological Data	D- 2
Presentation of Data	D- 3
Organization and Presentation of Biological Data and Reports	D- 4
Preparation of Survey Reports	D- 5
An Initiation into Statistics	D- 6
Administrative Need for Biological Information	D- 7
Dissemination of Technical Information to the Public	D- 8
Water Quality Surveys	D- 9
Application of Biological Data	D-10

THE INTERPRETATION OF BIOLOGICAL DATA WITH REFERENCE TO WATER QUALITY

I INTRODUCTION

Sanitary engineers like to have data presented to them in a readily assimilable form and some of them seem a little impatient with biologists who appear unable to provide definite quantitative criteria applicable to all kinds of water conditions. I think the feeling tends to be that this is the fault of biologists, and if they would only pull themselves out of the scientific stone-age all would be well. I will try to explain here why I believe that biological data can never be absolute nor interpretable without a certain amount of expertise. In this respect biologists resemble medical men who make their diagnoses against a complex background of detailed knowledge. Anyone can diagnose an open wound but it takes a doctor to identify an obscure disease; and although he can explain how he does it he cannot pass on his knowledge in that one explanation. Similarly, one does not need an expert to recognize gross organic pollution, but only a biologist can interpret more subtle biological conditions in a water body; and here again he can explain how he does it, but that does not make his hearer a biologist. Beck (1957) said something similar at a previous symposium in Cincinnati in 1956.

II THE COMPLEXITY OF BIOLOGICAL REACTIONS TO WATER CONDITIONS

A Complexity of the Aquatic Habitat

The aquatic habitat is complex and consists not only of water but of the substrata beneath it, which may be only indirectly influenced by the quality of the water. Moreover, in biological terms, water quality includes such features as rate of flow and temperature regime, which are not considered of direct importance by the chemist. To many animals and plants, maximum summer temperature or maximum rate of flow is just as important as minimum oxygen tension. The result is that inland waters provide an enormous array of different combinations of conditions, each of which has its own community of plants and animals; and the variety of species involved is very great. Thus, for example, Germany has about 6000 species of aquatic animals (Illies 1961a) and probably at least as many species of plants. Yet Europe has a rather restricted fauna because of the Pleistocene ice age; in most other parts of the world the flora and fauna are even richer.

B Distribution of Species and Environmental Factors

We know something about the way in which species are distributed in the various habitats, especially in the relatively much studied continent of Europe, but we have, as yet, little idea as to what factors or combination of factors actually control the individual species.

1 Important ecological factors

Thus, it is possible to list the groups of organisms that occur in swift stony upland rivers (rhithron in the sense of Illies, 1961b) and to contrast them with those of the lower sluggish reaches (potamon). Similarly we know, more or less, the different floras and faunas we can expect in infertile (oligotrophic) and fertile (eutrophic) lakes. We are, however, much less informed as to just what ecological factors cause these differences. We know they include temperature and its yearly amplitude; oxygen, particularly at minimal levels; plant nutrients, such as nitrate, phosphate, silica, and bicarbonate; other ions in solution, including calcium, chloride, and possibly hydrogen; dissolved organic matter, which is necessary for some bacteria and fungi and probably for some algae; the nature of the substratum; and current.

2 Complexity of interacting factors

We also know these factors can interact in a complex manner and that their action on any particular organism can be indirect through other members of the biota.

a Induced periphyton growths

Heavy growths of encrusting algae induced by large amounts of plant nutrients, or of bacteria induced by ample supplies of organic matter, can eliminate or decimate populations of lithophile insects by simple mechanical interference. But the change does not stop there: the growths themselves provide habitats for the animals, such as Chironomidae and Naidid worms, which could not otherwise live on the stones.

b Oxygen levels and depositing substrates

If oxygen conditions over a muddy bottom reach levels just low enough to be intolerable to leeches, tubificid worms, which the leeches normally hold in check, are able to build up to enormous numbers especially as some of their competitors (e.g. Chironomus) are also eliminated.

c Oxygen levels and non muddy substrates

One then finds the typical outburst of sludge worms, so often cited as indicators of pollution. This does not happen if the same oxygen tension occurs over sand or rock, however, as these are not suitable substrata for the worms. Many such examples could be given, but they would only be ones we understand; there must be a far greater number about which we know nothing.

d One must conclude, therefore, that quite simple chemical changes can produce far-reaching biological effects; that we only understand a small proportion of them; and that they are not always the same.

3 Classic examples

This seems like a note of despair, however, if water quality deviates too far from normal, the effects are immediately apparent. Thus, poisonous substances eliminate many species and may leave no animals (Hynes 1960); excessive quantities of salt remove all leeches, amphipods, and most insects and leave a fauna consisting largely of Chironomidae, caddis worms, and oligochaetes (Albrecht 1954) and excessive amounts of dissolved organic matter give rise to carpets of sewage fungus, which never occur naturally. Here no great biological expertise is needed, and there is little difficulty in the communication of results. It is when effects are slighter and more subtle that biological findings become difficult to transmit intelligibly to other disciplines.

III THE PROBLEMS IN PRESENTATION OF BIOLOGICAL RESULTS

Because of these difficulties various attempts have been made to simplify the presentation of biological findings, but to my mind none of them is very successful because of the complexity of the subject. Early attempts at systematization developed almost independently on the two sides of the Atlantic, although they had some similarities.

A Early Studies in the United States (Richardson and the Illinois River)

In America, there was a simple division into zones of pollution, e.g. degradation, septic, and recovery, which were characterized in broad general terms. This simple, textbook approach is summarized by Whipple et al. (1947), and serves fairly well for categorizing gross organic pollution such as has been mentioned above. It was, however, soon found by Richardson (1929) during his classical studies on the Illinois River that typical "indicators" of foul conditions, such as Tubificidae and Chironomus, were not always present where they would be expected to occur. This was an early indication that it is not the water quality itself that provides suitable conditions for "pollution faunas," but other, usually associated, conditions - in this instance deposits of rich organic mud. Such conditions may, in fact, be present in places where water quality in no way resembles pollution, e.g., upstream of weirs in trout streams where autumn leaves accumulate and decay and cause the development of biota typical of organically polluted water. Samples must therefore be judged against a background of biological knowledge. Richardson was fully aware of this and was in no doubt about the condition of the Illinois River even in places where his samples showed few or no pollution indicators.

B The European Saprobic System

In Europe, the initial stress was primarily on microorganisms and results were first codified in the early years of the century by Kolkwitz and Marsson. In this "Saprobiensystem," zones of organic pollution similar to those described by the American workers were defined and organisms were listed as characteristic of one or more zones;

TABLE I

SAPROBIENSYSTEM - A European system of classifying organisms according to their response to the organic pollution in slow moving streams. (22)

Alpha-Mesosaprobic Zone - Area of active decomposition, partly aerobic, partly anaerobic, in a stream heavily polluted with organic wastes.

Beta-Mesosaprobic Zone - That reach of stream that is moderately polluted with organic wastes.

Oligosaprobic Zone - That reach of a stream that is slightly polluted with organic wastes and contains the mineralized products of self-purification from organic pollution, but with none of the organic pollutants remaining.

Polysaprobic Zone - That area of a grossly polluted stream which contains the complex organic wastes that are decomposing primarily by anaerobic processes.

A recent exposition of this list is given by Kolkwitz (1950). It was then claimed that with a list of the species occurring at a particular point it was possible to allocate it to a saprobic zone. This system early met with criticism for several reasons. First,

TABLE 2

SAPROBICITY LEVELS ACCORDING TO THE TROPHIC STRUCTURE OF THE COMMUNITIES OF ORGANISMS

Saprobicity Level	Structure of the Communities of Organisms
I β-oligosaprobic	Balanced relationship between producers, consumers and destroyers; the communities of organisms are poor in individuals but there is a moderate variety of species, small biomass and low bioactivity.
II α-oligosaprobic	Balanced relationship between producers, consumers and destroyers; communities of organisms are rich in individuals and species with a large biomass and high bioactivity.
III β-mesosaprobic	Substantially balanced relationship between producers, consumers and destroyers; a relative increase in the abundance of destroyers and, accordingly, of the consumers living off them; communities of organisms are rich in individuals and species with a large biomass and high bioactivity.
IV α-mesosaprobic	Producers decline as compared with an increase in consumers and destroyers; mixotrophic and amphitrophic forms predominate among the producers; communities of organisms rich in individuals but poor in species with a large biomass and extremely high bioactivity; still only few species of macro-organisms; mass development of bacteria and bacteria-eating ciliates.
V β-polysaprobic	Producers drastically decline; communities of organisms are extremely rich in individuals but poor in species with a large biomass and high bioactivity; macrofauna represented only by a few species of tubificids and chironomids; as in IV these are in great abundance; mass development of bacteria and bacteria-eating ciliates.
VI α-polysaprobic	Producers are absent; the total biomass is formed practically solely by anaerobic bacteria and fungi; macro-organisms are absent; flagellates outnumber ciliates amongst the protozoa.

Saprobicity - "Within the bioactivity of a body of water, Saprobicity is the sum total of all those metabolic processes which are the antithesis of primary production. It is therefore the sum total of all those processes which are accompanied by a loss of potential energy." Part I, Prague Convention.

all the organisms listed occurred in natural habitats--they were not evolved in polluted water--and there was much doubt as to the placing of many of the species in the lists. The system, however, did serve to codify ecological knowledge about a long list of species along an extended trophic scale. Its weaknesses appeared to be merely due to lack of knowledge; such a rigid system took far too little account of the complexity of the reaction of organisms to their habitats. For instance, many organisms can be found, albeit rarely, in a wide range of conditions and others may occur in restricted zones for reasons that have nothing to do with water quality. We often do not know if organisms confined to clean headwaters are kept there by high oxygen content, low summer temperatures, or inability to compete with other species under other conditions. In the swift waters of Switzerland the system broke down in that some organisms appeared in more polluted zones than their position in the lists would indicate. Presumably here the controlling factor was oxygen, which was relatively plentiful in turbulent cold water. In a recent series of experiments, Zimmerman (1962) has proven that current alone has a great influence on the biota, and identically polluted water flowing at different speeds produces biotic communities characteristic of different saprobic levels. He finds this surprising, but to me it seems an expected result, for the reasons given above.

C Recent Advances in the Saprobic System

1 Perhaps Zimmerman's surprise reflects the deeply rooted entrenchment of the Saprobiensystem in Central Europe. Despite its obvious shortcomings it has been revised and extended. Liebmann (1951) introduced the concept of considering number as well as occurrence and very rightly pointed out that the community of organisms is what matters rather than mere species lists. But he did not stress the importance of extrinsic factors, such as current, nor that the system can only apply to organic pollution and that different types of organic pollution differ in their effects; e.g., carbohydrate solutions from paper works produce different results from those of sewage, as they contain little nitrogen and very different suspended solids. Other workers (Sladecek 1961 and references therein) have subdivided the more polluted zones, which now, instead of being merely descriptive, are considered to represent definite ranges of oxygen content, BOD, sulfide, and even E. coli populations. Every water chemist knows that BOD and oxygen content are not directly related and to assume that either should be more than vaguely related to the complexities of biological reactions seems to me to indicate a fundamental lack of ecological understanding. I also think it is damaging to the hope of mutual understanding between the various disciplines concerned with water quality to give the impression that one can expect to find a close and rigid relationship between water quality measurements as assessed by different sets of parameters. Inevitably these relationships vary with local conditions; what applies in a sluggish river in summer will certainly not apply to a mountain stream or even to the same river in the winter. Correlation of data, even within one discipline, needs understanding, knowledge, and judgment.

2 Caspers and Schulz (1960) showed that the failure of the system to distinguish between waters that are naturally productive and those artifically enriched can lead to absurd results. They studied a canal in Hamburg, which because of its urban situation can only be regarded as grossly polluted. Yet it develops a rich plankton,

the composition of which, according to the system, shows it to be virtually clean.

D Numerical Application of the Saprobic System

Once the Saprobiensystem was accepted it was logical to attempt to reduce its findings to simple figures or graphs for presentation of results. Several such methods were developed, which are described by Tumpling (1960), who also gives the original references. In all these methods, the abundance of each species is recorded on some sort of logarithmic scale (e.g. 1 for present, 3 for frequent, 5 for common, etc.). The sums of these abundances in each saprobic level are plotted on graphs, the two most polluted zones showing as negative and others as positive. Or, the various saprobic levels are given numerical values [1 for oligosaprobic (clean), 2 for β-mesosaprobic, etc.] and the rating for each species is multiplied by its abundance number. The sum of all these products divided by the sum of all the frequencies gives a "saprobic index" for the locality. Clearly the higher this number, the worse the water quality in terms of organic pollution. In a similar way the so-called "relative Belastung" (relative load) is calculated by expressing the sums of all the abundances of organisms characteristic of the two most-polluted zones as a percentage of the sum of all abundances. Then 100 percent is completely polluted water, and clean localities will give a low number.

E Weaknesses of the Saprobic System

There are various elaborations of these methods, such as sharing of species between zones and taking account of changes in base-line as one passes downstream. None of them, however, eliminates the basic weaknesses of the system nor the fact that, as Caspers and Schulz (1960) point out, there is little agreement between the various authors in the assignment of species to the different levels. Therefore, one gains a number or a figure that looks precise and is easily understood, but it is based on very dubious foundations.

F Comparative North American Systems

Similar systems are indigenous to North America, but were independently evolved.

1 Wurtz (1955) and Wurtz and Dolan (1960) describe a system whereby animals are divided into sensitive-to-pollution and non-sensitive (others are ignored), and also into burrowing, sessile, and foraging species (six classes).

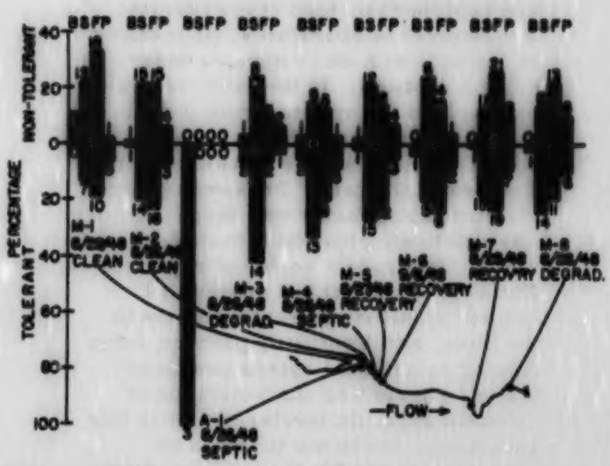


Figure 1. Histograms, based on selected organisms, illustrating stream reaches of clean, degradation, septic, and recovery conditions [after Wurtz] (22)

Numbers of these species represented are plotted for each station as six histograms on the basis of percentage of total number of species. If the constitution of the fauna from control stations or from similar localities is known, it is possible to express numerically "biological depression" (i.e., percentage reduction in total number of species), "biological distortion" (changes in proportions of tolerant and non-tolerant species), and "biological skewness" (changes in the ratios of the three habitat classes). Such results must, of course, be evaluated, and the definition of tolerance is quite subjective; but the method has the advantages of simplicity and dependence on control data. Like the Saprobiensystem, however, it can have no universal validity. It also suffers from the fact that it takes no account of numbers; a single specimen, which may be there by accident, carries as much weight as a dense population.

2 Patrick (1949) developed a similar system in which several clean stations on the water body being investigated are chosen, and the average number of species is determined occurring in each of seven groups of taxa chosen because of their supposed reaction to pollution. These are then plotted as seven columns of equal height, and data from other stations are plotted on the same scale; it is assumed that stations differing markedly from the controls will show biological imbalance in that the columns will be of very unequal heights. Number is indicated by double width in any column containing species with an unusual number of individuals. I have already questioned the usefulness of this method of presentation (Hynes 1960), and doubt whether it gives any more readily assimilable data than simple tabulation; it does however, introduce the concept of ecological imbalance.

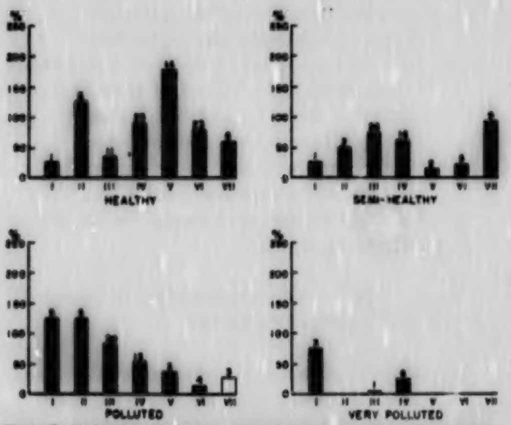


Figure 2. Histograms, based on selected organisms, illustrating healthy, semi-healthy, polluted, and very polluted stations in Conestoga Basin, Pa. [after Patrick] (22)

TABLE 3 —Classification of Groups of Organisms Shown in Figure 2

GROUP	ORGANISM
I	Blue-green algae; green algae of the genera *Stigeoclonium*, *Spirogyra*, and *Tribonema*; the bdelloid rotifers plus *Cephalodella megalocephala* and *Proales decipiens*
II	Oligochaetes, leeches, and pulmonate snails
III	Protozoa
IV	Diatoms, red algae, and "most of the green algae"
V	All rotifers not included in Group I, clams, gill-breathing snails, and tricladid flatworms
VI	All insects and crustacea
VII	All fish

3 Beak (1964), another author, recognized the need for a concise expression of pollution based on biological information. Toward this end, he developed a method of biological scoring which is based on the frequency of occurrence of certain macroscopic invertebrates obtained from 6 years of study on one river. It will be noted that the Biological Score is a modification and expansion of Beck's Biotic Index.

The indicator organisms are divided into three categories: Group I contains the pollution-tolerant species; Group II comprises those species which are facultative with respect to pollution; and Group III contains the pollution-intolerant forms. Each group is assigned a weighted score that can be allotted to field samples on the following basis:

a Normal complement of Group III scores 3 points.

b Normal complement of Group II scores 2 points.

c Normal complement of Group I scores 1 point.

The scores are additive; thus an unpolluted stream will have a Biological Score of 6. If only pollution-tolerant forms are found, the score will be 1. If no organisms are found, the score will be zero. Furthermore, a score of, 1 or 2 points could be allotted to Group III when less than the normal complement is present. Group II could be treated in a similar manner. This scoring device correlated well with the biological oxygen demand, dissolved oxygen, and solids content of the receiving water. Beak also related his scoring device to the fisheries potential. This relationship is shown in Table 4]

TABLE 4

TENTATIVE RELATIONSHIP OF THE BIOLOGICAL SCORE TO THE FISHERIES POTENTIAL (after Beak, 1964) (30)

Pollution status	Biotic index	Fisheries potential
Unpolluted	6	All normal fisheries for type of water well developed
Slight to moderate pollution	5 or 4	Most sensitive fish species reduced in numbers or missing
Moderate pollution	3	Only coarse fisheries maintained
Moderate to heavy pollution	2	If fish present, only those with high toleration of pollution
Heavy pollution	1	Very little, if any, fishery
Severe pollution, usually toxic	0	No fish

4 It has long been known that ecologically severe habitats contain fewer species than normal habitats and that the few species that can survive the severe conditions are often very abundant as they lack competitors. Examples of this are the countless millions of Artemia and Ephydra in saline lakes and the Tubifex tubifex in foul mud. This idea has often been expressed in terms of diversity, which is some measure of numbers of species divided by number of specimens collected. Clearly, such a parameter is larger the greater the diversity, and hence the normality of the habitat. Unfortunately, though as the number of species in any habitat is fixed, it also decreases as sample size increases so no index of diversity has any absolute value (Hairston 1959). If a definite sample size is fixed, however, in respect to numbers of organisms identified, it is possible to arrive at a constant index.

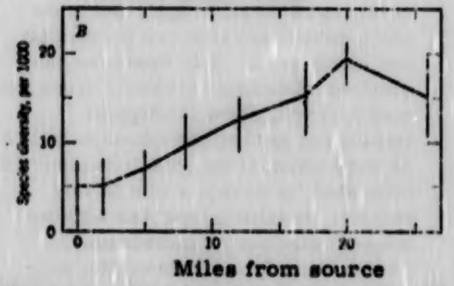


Figure 3. Zooplankton species diversity per thousand individuals encountered in marine systems affected by waste waters from petrochemical industrial wastes. The vertical lines indicate seasonal variations. (30)

5 Patrick et al. (1954) in effect used this concept in a study of diatom species growing on slides suspended in water for fixed periods. They identified 8000 specimens per sample and plotted the results as number of species per interval against number of specimens per species on a logarithmic scale. This method of plotting gives a truncated normal curve for a wide variety of biotic communities. In an ordinarily diverse habitat the mode is high and the curve short; i.e., many species occur in small numbers and none is very abundant. In a severe habitat the mode is low and the curve long; i.e., there are few rare species and a few with large numbers. This, again, seems to me to be an elaborate way of presenting data and to involve a lot of unnecessary arithmetic.

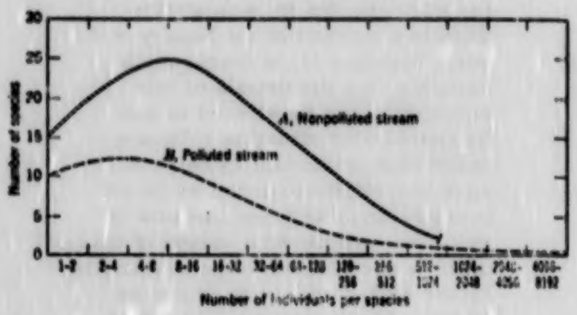


Figure 4. A graphic comparison of diatom communities from two different environments. (After Patrick et al., 1954) (30)

6 Diversity indices vs tabulated data

Allanson (1961) has applied this method to the invertebrate faunas of streams in South Africa and has shown, as has Patrick for diatoms, that the log normal curve is flatter and longer for polluted stations; the difference, however, is not so apparent that it does not need exposition. Here, again, I would suggest that tabulated data are just as informative. Indeed I would go further and say that tabulated data are essential in the present state of our knowledge. We are learning as we go along and if the details of the basic findings are concealed by some sort of arithmetical manipulation they cannot be re-interpreted in the light of later knowledge, nor are they preserved in the store of human knowledge. This point becomes particularly clear when one examines some of the early studies that include tables. Butcher (1946) requotes a considerable amount of data he collected from studies of various English rivers during the thirties; they are not only clear and easy to follow, but they are also informative about the generalities of pollution in a way that data quoted only within the confines of some particular system are not.

7 Examples of tabulated data (Table 5)

Simple tabulation of biological data in relation to water quality, either in terms of number of organisms, percentage composition of the biota, some arbitrary abundance scale, or as histograms, has been effectively practiced in many parts of the world: in America (Gaufin and Tarzwell 1952, Gaufin 1958), Africa (Harrison 1958 and 1960, Hynes and Williams 1962), Europe (Albrecht 1954, Kaiser 1951, Hynes 1961, Hynes and Roberts 1962), and New Zealand (Hirsch 1958) to cite a few. These tabulated data are easy to follow, are informative to the expert reader, and conceal no facts. Although the non-biologist may find them tedious, he need only read the explanatory paragraphs. It is a delusion to think that it is possible to reduce biological data to simple numerical levels. At best, these can only be produced for limited situations and

TABLE 5

ORGANISM	1 [illegible] left	 mid	 right	2 [illegible] left	 mid	 right	3 [illegible] left	 mid	 right
[illegible]	F								
[illegible]	C								F
Spongilla fragilis								A	
Trochospongilla leidyi	C			C					
Unidentified Sponge					F			A	
Cordylophora lacustris		F		F	A	A			
Dugesia tigrina	3		2	4	4	6	19		19
Urnatella gracilis	C	C	C	C	F		F		F
Paludicella articulata	C			F	F	F	F		F
Fredericella sultana	C								
Pristina							1		
Nais communis				1		1		7	
Paranais									
Unidentified leech									1
Unidentified Beetle			1						
Chaoborus punctipennis					1				
Hydrobaenus sp. A	31	47	5	9	117	68	1	26	4
Cricotopus bicinctus						1			
Unidentified Tendipedini								1	
Harnischia sp. A		3	2		20	8	1	8	
Tendipes nervosus	1				4	15		2	1
Tendipes modestus	20	14	16	7	12	7	1		3
Polypedilum sp. B									1
Calopsectra exigua			2	3	1	48	3	169	101
Tricorythodes	1			1			4		1
Stenonema				1					
Agraylea			2				1		
Athripsodes				2					
Potamyia flava				42		44	18	131	27
Hydropsyche orris				161		17	7	1	11
Cheumatopsyche		1		4	3	41		10	17
Psychomyiidae Genus A	51	27	21	10	15	22	11	103	21
Lithasia verrucosa									1
Ferrissia shimekii				4		33	7	5	19
Quadrula sp.						1	1		
Quadrula tuberculata						2			
Corbicula fluminea	4	1	5	41		1	77		7
TOTAL	152	[illegible]	56	251	[illegible]	161	161	373	437
	[illegible]			671			1,151		

F - few C - common A - abundant

Benthos from Pickwick Tailwater (35)

even then they need verbal exposition; at worst, they give a spurious impression of having absolute validity.

8 Comparison of stations

My final point in this section concerns comparisons. It is claimed that the German system, in effect, measures an absolute state, a definite level of water quality. We have seen that this is not a tenable claim. In the other systems, by and large, the need to establish local control stations at which to measure the normal or "natural" biotic conditions is accepted, and then other areas are compared with this supposed norm. This is, of course not always possible as there may remain no unaffected area, or no unaffected area that is, with respect to such factors as current, nature of substratum, etc., sufficiently similar to act as a base-line for data. Nevertheless, basically, these systems can be used to compare stations and thus to assess changes in water quality. In doing this, they can all be used more or less successfully, but I maintain that a table is just as useful as an elaborate analysis, and I believe that the table should be included with whatever is done. For a particular situation, however, it is often possible to distill the data into a single figure as a measure of similarity between stations.

9 Coefficients of similarity

Burlington (1962) and Dean and Burlington (1963) have recently proposed an entirely objective means of doing this, which involves simple arithmetical manipulation. In his system, a "prominence value" is calculated for each species at each station. This is a product of its density and some function of its frequency in samples, but the details of this calculation can be altered to suit any particular situation. Then a coefficient of similarity between each pair of stations can be calculated by dividing twice the sum of the [illegible] ver prominence values of taxa that the two stations have in common by the sum of all the prominence values of both stations. Identical stations will then have a coefficient of similarity of 1:00; this coefficient will be lower the more the stations differ from one another. This is an easy way to compare stations in an entirely unbiased way and as such may satisfy the need for numerical exposition; however, it tells one nothing about why the localities are different and like all the other more or less numerical methods of presenting data has no absolute value. Moreover, it still leaves unanswered the fundamental question of how different is "different?"

TABLE 6

BIOTIC INDEX[1]

Clean

WATER QUALITY (high multiple use indicated)

Organisms in Order of Tendency to Disappear as Degree of Pollution Increases

Polluted

Types of Organisms Present	Variety Present	✓Total Number of Groups Present				
		0-1	2-5	6-10	11-15	16+
Plecoptera nymph present	More than one species	—	Bio 7	Index 8	9	10
	One species only	—	6	7	8	9
Ephemeroptera nymph present	More than one species*	—	6	7	8	9*
	One species only*	—	5	6	7	8
Trichoptera larvae present	More than one species•	—	5	6	7	8
	One species only•	4	4	5	6	7
Gammaridae present	All above species absent	3	4	5	6	7
Asellus and/or Lirceus present	All above species absent	2	3	4	5	6
Tubificid worms, Tendipes, and Cricotopus bicinctus (one or more of these groups)	All above species absent	1	2	3	4	—
All above types absent	Some organisms such as Eristalis tenax not requiring dissolved oxygen may be present	0	1	2	--	--

*Stenonema nepotellum excluded

10* main stream reservoirs and west Tennessee streams

• Stenonema nepotellum (Ephem.) is counted in this section for the purpose of classification.

✓ ONE FOR EACH KNOWN SPECIES IN THESE GROUPS:

Platyhelminthes
Hirudinea
Mollusca
Crustacea
Plecoptera
Diptera (excluding specific ones listed below)
Coleoptera
Neuroptera

✓ ONE FOR EACH GROUP, REGARDLESS OF NUMBER OF SPECIES, ETC.:

Annelida excluding Naididae
Naididae
Each Mayfly genera (excluding Stenonema nepotellum)
Stenonema nepotellum
Each Trichoptera family
Chironomidae (excluding specific ones listed below)
Chironomus riparius and plumosus and Cricotopus bicinctus.
Family Simuliidae

[1] adapted from Trent River Board - Tennessee Stream Pollution Control Board 8/66 RMS

IV THE PROBLEMS OF SAMPLING

The systems outlined above are all based on the assumption that it is possible to sample an aquatic habitat with some degree of accuracy; this is a dubious assumption, however, when applied to biological data. From what has been said about the complexity of biological reactions to the various factors in the environment, and from the obvious fact that rivers especially are a mosaic of microhabitats, it is clear that to achieve numerical accuracy or even some limits of confidence considerable numbers of samples need to be taken. Indeed, even in so apparently unvaried a habitat as a single riffle, Needham and Usinger (1956) showed that a very large number of samples would be necessary to give significant numerical data.

A Representative Sampling

There is a limit to the number of samples that can reasonably be taken and, anyway, it is desirable to sample many different types of habitat so as to get as broad as possible an estimate of the biota. This is the more recent approach of most of the workers in Central Europe, who have been content to cite abundances on a simple relative but arbitrary scale and to convert this to figures on some sort of logarithmic scale for use in calculations. An alternative is to express the catch in terms of percentage composition, but this has the disadvantage that micro- and macro-organisms cannot be expressed on the same scale as they are obtained by different collecting techniques. Also, of course, implicit in this approach is the assumption that the sampling is reasonably representative. Here again we run into the need for knowledge and expertise. In collection as well as in interpretation, the expert is essential. Biological sampling, unlike the simple, or fairly simple, filling of bottles for chemical analysis or the monitoring of measuring equipment, is a highly skilled job and not one to be handed over to a couple of vacationing undergraduates who are sent out with a Surber sampler and told to get on with it. This point has also been made by other biologists, e.g., Patrick (1961) who stresses the need for skilled and thorough collecting even for the determination of a species list.

B Non-Taxonomic Techniques

Alternatively we can use the less expert man when concentrating on only part of the habitat, using, say, microscopical slides suspended in the water to study algal growth. This method was extensively used by Butcher (1946), and Patrick et al. (1954) who studied diatoms in this way. This gives only a partial biological picture, but is useful as a means of monitoring a stretch of water where it is possible that changes might occur. It is a useful short-hand method, and as such is perhaps comparable to studying the oxygen absorbed from potassium permanganate instead of carrying out all the usual chemical analyses on water. A short method of this kind may serve very well most of the time, but, for instance, would not be likely to detect an insecticide in concentrations that could entirely eliminate arthropods and hence fishes by starvation.

C Monitoring

It is possible to work out biological monitoring systems for any specific purpose. The simplest of these is the cage of fish, which, like a single type of chemical analysis, can be expected to monitor only one thing -- the ability of fish to live in the water -- with no information on whether they can breed or whether there is anything for them to eat. Beck et al. (1959) describe a neat way in which the common constituents of the bottom fauna of Lake Ontario can be used to monitor the effluents from an industrial site. Obviously there is much room for such ingenuity in devising biological systems for particular conditions, but this is perhaps outside the scope of this meeting.

V CONCLUSIONS

It may appear from the previous sections that my attitude to this problem is entirely obstructionist. This is far from being so. Water quality is as much biological phenomenon as it is a chemical or physical one; often what we want to know about water is almost exclusively biological -- will it smell nasty, is it fit to drink, can one bathe in it, etc? I suggest, therefore, that it is desirable to organize water monitoring programs that will tell one what one wants to know. There is no point in measuring everything biological, just as there is no point in performing every possible chemical analysis; what is measured should be related to local conditions. It would be a waste of time to measure oxygen content in a clean mountain stream; we know it to be high, and it becomes worth measuring only if we suspect that it may have been lowered by pollution. Similarly, there is little point in studying the plankton in such a stream; we know it only reflects the benthic flora. In a lake or in a slow river, on the other hand, if our interest in the water lies in its potability, records of the plankton are of considerable importance as changes in plankton are, in fact, changes in the usability of the water.

A Periphyton and Benthos Studies

For long-term studies, especially for the recording of trends or changes induced by pollution, altered drainage, agricultural poisons, and other havoc wrought by man, one can expect informative results from two principal techniques: First, we can study microscopic plant and animal growth with glass slides placed in the water for fixed periods; second, we can obtain random samples of the benthic fauna. The algae and associated microfauna tell one a good deal about the nutrient condition of the water and the changes that occur in it, and the larger benthic fauna reveal changes in the trophic status, siltation due to soil erosion, effects of insecticides and other poisons, etc.

B Varying Levels of Complexity

The study of growths on glass slides is reasonably skilled work, but can easily be taught to technicians; like chemical monitoring, such study needs to be done fairly often. Sampling the benthos is more difficult and, as explained above, needs expert handling; unlike most other monitoring programs, however, it need be done only infrequently, say, once or twice a year. Inevitably sampling methods will vary with type of habitat; in each case, the question will arise as to whether it is worth looking at the fish also. It is here that the biologist must exercise judgment in devising and carrying out the sampling program.

C Data Interpretation

Judgment is also needed in the interpretation of the data. It is for this reason I maintain that it should all be tabulated so that it remains available for reassessment or comparison with later surveys. If need be, some sort of numerical format can be prepared from the data for ad hoc uses, but it should never become a substitute for tabulations. Only in this way can we go on building up our knowledge. Perhaps some day we shall be able to pass all this information into a computer, which will then be able to exercise better judgment than the biologist. I hope this will happen, as computers are better able to remember and to cope with complexity than men. It will not, however, pension off the biologist. He will still be needed to collect and identify the samples. I cannot imagine any computer wading about on rocky riffles nor persuading outboard motors and mechanical grabs to operate from the unstable confines of small boats. We shall still need flesh and blood biologists long after the advent of the hardware water chemist, even though, with reference to my earlier analogy, a Tokyo University computer recently outpointed 10 veteran medicals in diagnosing brain tumors and heart disease. It should be pointed out, however, that the computer still had to be fed with information, so we are still a long way from the hardware general practitioner. I believe though that he is likely to evolve before the hardware biologist; after all, he studies only one animal.

REFERENCES

1 Albrecht, M. L. Die Wirkung der Kaliabwasser auf die Fauna der Werra and Wipper. Z. Fisch. N. F. 3:401-26. 1954.

2 Allanson, B. R. Investigations into the ecology of polluted inland waters in the Transvaal. Part I. Hydrobiologia 18:1-94. 1961.

3 Bartsch, A. F. and Ingram, W. M. Biological Analysis of Water Pollution in North America. Verh. Internat. Verein. Limnol. 16:786-800. 1968.

4 Beak, T. W., de Courval, C. and Cooke, N. E. Pollution monitoring and prevention by use of bivariate control charts. Sew. Industr. Wastes 31:1383-94. 1959.

5 Beck, W. M., Jr. The Use and Abuse of Indicator Organisms. Transactions of a Seminar on Biological Problems in Water Pollution. Cincinnati. 1957.

6 Burlington, R. F. Quantitative Biological Assessment of Pollution. J. Wat. Poll. Contr. Fed. 34:179-83. 1962.

7 Butcher, R. W. The Biological Detection of Pollution. J. Inst. Sew. Purif. 2:92-7. 1946.

8 Cairns, John, Jr. et al. A Preliminary Report on Rapid Biological Information Systems for Water Pollution Control. JWPCF. 42(5):685-703. 1970.

9 Caspers, H. and Schulz, H. Studien zur Wertung der Saprobiensysteme. Int. Rev. ges. Hydrobiol. 45:535-65. 1960.

10 Dean, J. M. and Burlington, R. F. A Quantitative Evaluation of Pollution Effects on Stream Communities. Hydrobiologia 21:193-9. 1963.

11 Ferdjingstad, E. Taxonomy and Saprobic Valency of Benthic Phytomicroorganisms. Inter. Revue der Ges. Hydrobiol. 50(4):475-604. 1965.

12 Ferdjingstad, E. Pollution of Streams Estimated by Benthal Phytomicroorganisms. I. A System Based on Communities of Organisms and Ecological Factors. Int. Revue ges. Hydrobiol. 49:63-131.

13 Gaufin, A. R. The Effects of Pollution on a Midwestern Stream. Ohio J. Sci. 58:197-208. 1958.

14 Gaufin, A. R. and Tarzwell, C. M. Aquatic Invertebrates as Indicators of Stream Pollution. Pub. Hlth. Rep. 67:57-64. 1952.

15 Hairston, N. G. Species Abundance and Community Organization. Ecology 40:404-15. 1959.

16 Harrison, A. D. The Effects of Sulphuric Acid Pollution on the Biology of Streams in the Transvaal, South Africa. Verh. Int. Ver. Limnol. 13:603-10. 1958.

17 Harrison, A. D. The role of River Fauna in the Assessment of Pollution. Cons. Sci. Afr. Sud Sahara Pub. 64:199-212. 1960.

18 Hirsch, A. Biological Evaluation of Organic Pollution of New Zealand Streams. N.Z. J. Sci. 1:500-53. 1958.

19 Hynes, H. B. N. The Biology of Polluted Waters. Liverpool. 1960.

20 Hynes, H. B. N. The Effect of Sheep-dip Containing the Insecticide BHC on the Fauna of a Small Stream. Ann. Trop. Med. Parasit. 55:192-6. 1961.

21 Hynes, H. B. N. and Roberts, F. W. The Biological Effects of Detergents in the River Lee, Hertfordshire. Ann. Appl. Biol. 50:779-90. 1962.

22 Hynes, H. B. N. and Williams, T. R. The Effect of DDT on the Fauna of a Central African Stream. Ann. Trop. Med. Parasit. 56:78-91. 1962.

23 Illies, J. Die Lebensgemeinschaft des Bergbaches. Wittenberg-Lutherstadt. 1961a.

24 Illies, J. Versuch einer allgemeiner biozonotischen Gliederung der Fliessgewasser. Int. Rev. ges Hydrobiol. 46:205-13. 1961b.

25 Ingram, W. M., Mackenthun, K. M., and Bartsch, A. F. Biological Field Investigative Data for Water Pollution Surveys. USDI, FWPCA Pub. WP-13, 139 pages. 1966.

26 Kaiser, E. W. Biolgiske, biokemiske, bacteriologiske samt hydrometriske undersogelser af Poleaen 1946 og 1947. Dansk. Ingenforen. Skr. 3:15-33. 1951.

27 Keup, Lowell E., Ingram, W. M., and Mackenthun, K. M. Biology of Water Pollution. USDI. FWPCA CWA-3, 290 pages. 1967.

28 Kolkwitz, R. Oekologie der Saprobien. Uber die Beziehungen der Wasserorganismen zur Ummelt. Schr. Reihe ver Wasserhyg. 4:64 pp. 1950.

29 Liebmann, H. Handbuch der Frischwasser und Abwasserbiologie. Munich. 1951.

30 Maciel, Norma C. Levantamento hipotético de um rio com rêde Surber. Inst. de Engenharia Sanitária, Rio de Janeiro, Brazil. Pub. No. 56, 96 pages. 1969. (Zones of pollution in a Brazilian river.)

31 Mackenthun, K. M. The Practice of Water Pollution Biology. USDI. FWPCA. 281 pp. 1969.

32 Needham, P. R. and Usinger, R. L. Variability in the Macrofauna of a Single Riffle in Prosser Creek, California, as indicated by the Surber Sampler. Hilgardia 24:383-409. 1956.

33 Olson, Theodore A., and Burgess, F. J. Pollution and Marine Ecology. Interscience Publishers. 364 pages. 1967.

34 Patrick, R. A Proposed Biological Measure of Stream Conditions, based on a Survey of the Conestoga Basin, Lancaster County, Pennsylvania. Proc. Acad. Nat. Sci. Phila. 101:277-341. 1949.

35 Patrick, R. A Study of the Numbers and Kinds of Species found in Rivers in Eastern United States. Proc. Acad. Nat. Sci. Phila. 113:215-58. 1961.

36 Patrick, R., Hohn, M. H. and Wallace, J. H. A New Method for Determining the Pattern of the Diatom Flora. Not. Nat. Phila. Acad. Sci. 259. 12 pp. 1954.

37 Patrick, Ruth. Benthic Stream Communities. Amer. Sci. 58:546-549. 1970.

38 Richardson, R. E. The Bottom Fauna of the Middle Illinois River, 1913-1925; Its Distribution, Abundance, Valuation and Index Value in the Study of Stream Pollution. Bull. Ill. Nat. Hist. Surv. 17:387-475. 1929.

39 Sinclair, Ralph M., and Ingram, William M. A New Record for the Asiatic Clam in the United States--The Tennessee River. Nautilus 74(3):114-118. 1961. (A typical benthos faunal list for a large inland unpolluted river, with an eroding substrate.)

40 Sladecek, Vladimir. Water Quality System. Verh. Internat. Verein. Limnol. 16:809-816. 1966.

41 Sladecek, V. Zur biologischen Gliederung der hoheren Saprobitatsstufen. Arch. Hydrobiol. 58:103-21. 1961.

42 Sladecek, Vladimir. The Ecological and Physiological Trends in the Saprobiology. Hydrobiol. 30:513-526. 1967.

43 Tumpling, W. V. Probleme, Methoden und Ergenbnisse biologischer Guteuntersuchungen an Vorflutern, dargestellt am Beispiel der Werra. 45:513-34. 1960.

44 Whipple, G. C., Fair, G. M. and Whipple, M. C. The Microscopy of Drinking Water. New York. 1947.

45 Woodiwiss, F. S. The Biological System of Stream Classification used by the Trent River Board. Chem. and Ind., pp. 443-447. March 1964.

46 Wurtz, C. B. and Dolan, T. A Biological Method Used in the Evaluation of Effects of Thermal Discharge in the Schuylkill River. Proc. Ind. Waste Conf. Purdue. 461-72. 1960.

47 Zimmerman, P. Der Einfluss auf die Zusammensetzung der Lebensgemeinschaften in Experiment. Schweiz. Z. Hydrol. 24:408-11. 1962.

48 Hynes, H. B. N. The Ecology of Flowing Waters in Relation to Management. JWPCF. 42(3):418-424. 1970.

49 Hynes, H. B. N. The Ecology of Running Waters. Univ. of Toronto Press. 555 pp. 1970.

50 Scott, Ralph D. The Macro-invertebrate Biotic Index - A Water Quality Measurement and Natural Continuous Stream Monitor for the Miami River Basin. 17 pp. The Miami Conservancy District, Dayton, OH 45402. 1969.

51 Cooke, Norman E. Stream Surveys Pinpoint Pollution. Industrial Water Engineering. p. 31-33. Sept. 1970.

This outline was prepared by Dr. H. B. N. Hynes, Chairman, Department of Biology, University of Waterloo, Ontario, Canada.

Reprinted from: Symposium Environmental Measurements Valid Data and Logical Interpretation, July 1964, PHS Publication No. 999-AP-15, pp. 289-298.

Figures, tables, additional references, and headings are editorial changes by Ralph Sinclair, Aquatic Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

SIGNIFICANCE AND INTERPRETATION OF BIOLOGICAL DATA

I INTRODUCTION

Recent books contain a vast array of designs for problems that are susceptible to experiment in such fields as agriculture, industrial chemistry and quality control, and medicine; but which of these are useful in limnological research or biological problems in water pollution control. Fundamental questions are now being asked by some people regarding the analytical part of experimenters' statistics - the statistical arithmetic. Not considering the widespread misuse of this arithmetic by investigators who have learned it from statistical "cookbooks," what does the arithmetic really tell us, even when it is applied in the way that its inventor intended? If investigators were really aware of the assumptions underlying the commonest statistical tests and estimates, would they continue to use these methods?

In order to reveal some of the traps into which aquatic biologists and others have fallen, ten sets of questions are presented to show what people have done to avoid these traps and to emphasize the danger of trusting to statistical arithmetic as protection. These questions were presented in "notes from a Laboratory of Medical Statistics" by personnel of the Department of Medical Statistics, New York University College of Medicine, 550 First Avenue, New York, New York 10016. Some of the questions have been modified by the outline author in order to relate them to biological problems in water pollution control.

II GENERAL STATISTICAL APPLICATIONS TO BIOLOGICAL STUDIES

A Questions for Use in Planning Investigations and in Evaluating Reports

1 Purpose and general method

a What is the immediate purpose of our investigation? That is, what are the specific questions to be answered by it?

b What is the remote (more general or ultimate) purpose of our investigation?

c Will the investigation be by an experiment in the strict sense? a survey? both experiment and survey?

d If the investigation is a survey, will it be anecdotal or evaluational?

e If the investigation is a survey, will it be retrospective or prospective, or a mixture of both?

f If the investigation is a survey, what is the simple outline of an imaginary experiment which, if it were possible, we would perform instead of the survey?

2 The population and sampling

a What is the initial definition of our population or universe, i.e., the kind of material to be studied and to which our conclusions will be applicable?

b What other populations are excluded by the definition given in response to Question a.

c If all the experimental units of our chosen population are not to be investigated, how is the sample to be taken?

d If the sample is not to be taken by a strictly automatic random process, how may the sampling method affect the definition of the population?

3 Subdivision of population (stratification)

a If we intend to subdivide (stratify) our population and test the factors under study (A, B, etc.) separately in samples from each subpopulation, what do we hope to gain thereby?

b What, in the form of a list, are the factors (in addition to factors under test) that affect, or could conceivably affect, the phenomena to be observed?

c Which of the factors listed in Question b are to be used in stratification?

d Why are the other factors listed in answer to Question b not to be used in stratification?

e Why is the stratification not to be coarser or finer?

f Are we going to make paired comparisons, i.e., within the same experimental unit, or between experimental units that are matched in some way? If so, why?

4 Skeleton tables for results

a How, in skeleton form, will the results appear?

b Are measurements to be presented as mensuration data (e.g., as means and variations) or as enumeration data (e.g., in contingency tables)? What will determine between these methods of presentation?

c What do we expect to be the order of magnitude of the frequencies and measurements (e.g., means of measurements, variations between measurements)?

d Are the sampling units correct?

e Are the denominators correct?

f How may the structure of our measurements mislead us?

5 Interpretation of results

a How shall we decide whether the results prove, or fail to prove, that the factors under test were responsible for the differences in outcome in the samples we compared - by inspection of the results in the light of our previous experience (e.g., experience of responses of organisms) or by a formal test of "statistical significance"?

b If the inspection method is used, what specifically (numerically) is the experience or background knowledge?

c If a formal test of "significance" is proposed and the investigation is an experiment, what precautions will be taken in order to make possible an inference of the form "either chance or the factors under test"?

d If the investigation is a survey, what would a test of significance tell us? Would it be helpful?

e If a significance test is to be applied, what does it mean in terms of an experiment conducted with chance? What assumptions underlie the test? What is the evidence that they are valid for our data?

f If a significance test is to be applied, what level of significance will be adopted as the critical level? Why was it chosen?

g What are the various possible outcomes of our investigation? What would be our interpretation and our action - our next step - after each outcome?

h How shall we estimate what our results really tell us, numerically, about the population represented by our samples?

i Do we intend, after the investigation, to search in the data for factors, effects or relationships other than those that we contemplated when we designed the investigation? If so, what risk do we run?

6 Nonrandom selection methods

a If the investigation is a survey, how will the samples for comparison (A, B, etc.) be selected?

b If the investigation is a survey, what will be the known or conceivable difference between the samples labeled A, B, etc., in addition to the factors of features that these letters represent?

c How shall we decide when to stop the investigation?

7 Risks of bias from selection rates

a What could conceivably make the selection rates of experimental units differ from each other?

b Is there any assurance the competition between selection rates will not lead to bias?

c If, in spite of the risks of bias from selection rates and other inevitable uncertainties in surveys, we intend to conduct a survey, what is our justification for doing so?

8 Sample sizes

a What sample sizes will be used in each group (A, B, etc.)?

b Will the samples be large enough to answer our questions?

c Will our facilities (personnel, time, money, etc.) suffice to produce the amount of information that we need?

9 Collecting the data

a Who? (persons, i.e., observers and others involved in the investigation)

b Where? (location and environment)

c When? (time relationships)

d How? (methods)

e When the possibility of bias is suggested by any of the preceding four questions, will the risk be removed, e.g., by standardization of techniques or controlled by randomization?

10 Losses and rejections

a What can we confidently expect to be the percentage completeness of our samples at the beginning and throughout the investigation?

b How are we going to insure that the percentage completeness of our samples will be as high as possible?

c What numerical effects would the anticipated numbers of lost experimental units have on our conclusions?

d Will records be made of all experimental units which, according to our plan ought to have been in our samples, but which were omitted at the start or were lost later? Will the records give reasons for each omission?

e What will be the effects of missing items of data in past records and of observations omitted during the investigation?

f Do we expect to reject any observations? If so, on what grounds? Could these rejections introduce bias?

B Sampling in Biological Studies - What are the factors, from a statistical point of view, that determine how large a sample is needed to get an answer to the problem?

III SPECIFIC STATISTICAL APPLICATION TO BIOLOGICAL STUDIES

A Statistical Criterion for Evaluating the Efficiency of Benthic Sampling Devices

1 Using standard benthic samplers, how many samples are needed to get an adequate picture of the benthic macroinvertebrates in a stream?

2 What is the value of artificial substrate samplers in biological stream studies?

3 Risks of bias from selection rates - an example of the Markov Chain.

B Biological Parameters and Their Value as Variables in Factor Analysis on Stream Pollution

C The Value of Correlation Analysis in Comparing Some Objective Biological Parameters

D Evaluation of an Experiment Designed to Show the Influence of Nutrient Concentration on the Quality of Pigments of Algae

REFERENCES

1 Castellvi, J. Un sencillo experimento para demostrar la influencia de la concentración de elementos nutritivos sobre la calidad de los pigmentos de las algas. Inv. Pesq. 25:157-160. 1964.

2 Gaufin, A.R., Harris, E.K. and Walters, H.J. A Statistical Evaluation of Stream Bottom Sampling Data Obtained from Three Standard Samplers. Ecology 37 (4):643-648. 1956.

2 Hynes, H.B.N. The Interpretation of Biological Data with Reference to Water Quality, pp. 289-298. In Symposium, Environmental Measurement. Valid Data and Logical Interpretation, USPHS No. 999-WP-15. 1964.

4 Margalef, R. Ecological Correlations and the Relationship Between Primary Productivity and Community Structure. In C.R. Goldman (ed.), Primary Productivity in Aquatic Environments. Mem. Ist. Ital. Idrobiol., 18 Suppl., Univ. Calif. Press, Berkeley. pp. 355-364. 1965.

5 Odum, E.P. Relationships Between Structure and Function in the Ecosystem. Japanese Journal of Ecology 12(3):108-118. 1962.

6 Shoji, H., Yamamoto, T. and Nakamura, T. Factor Analysis on Stream Pollution of the Yodo River System. Air and Wat. Pollut. Int. J. 10:291-299. 1966.

This outline was prepared by W. R. Duffer, Ph.D., Research Aquatic Biologist, Biological Sciences Section, Research Program, Robert S. Kerr Water Research Center.

PRESENTATION OF DATA

I INTRODUCTION

The data collected during any water quality survey must ultimately be reduced to some simple, expressive form for presentation. Ideally, the data presentation should be easily understood by the least trained personnel as well as by the trained professional. The data presentation alone may determine the response to the survey.

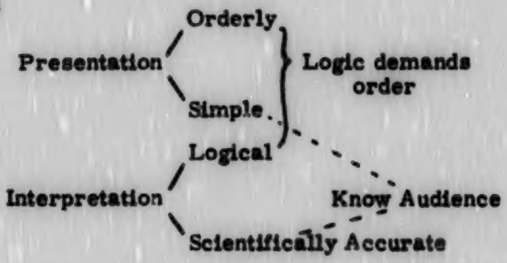
II ANALYSIS OF DATA

A Before one analyzes any information, the data must be set into logical order and the analyst must present the information to himself.

In developing this order, the analyst should keep in mind the objectives of the survey, and the variables encountered during the study.

The analyst's "private" presentation to himself enables him to evaluate or interpret the data in a logical and scientific manner.

B



III PRESENTATION OF DATA

This is one of the most important areas of report writing and unfortunately is often neglected or overlooked.

A Common Methods of Presenting Data

1 Single value

2 Single value plus related information

3 Graphic methods

B Single Value Approach

1 Methods commonly used

a Arithmetic mean

b Geometric mean

c Median value

d Mode value

2 Advantages of using single value approach

a Economical - engineering design criteria is often based on average values. In some instances minimum and maximum values are more critical.

b Instrumentation - advanced analytical techniques and instrumentation are accounting for the collection of tremendous amounts of information. The tendency is to compile this information on computers and use sophisticated statistical methods to arrive at representative mean values.

c Audience - since some readers (local, state or federal legislators) do not have the technical background or (especially), interest to read through many pages of numbers, it becomes convenient to summarize the numerous observations by using single representative values. In effect, the analyst is a salesman, his report being his product.

C Single Value Plus Related Information

1 Methods commonly used

a Single value plus maximum or minimum value

b Single value plus percentage range (usually 80%) of survey or laboratory results

c Single value plus standard deviations from the mean (depending upon degree of confidence required)

d Distribution and frequency distribution tables

2 Advantages of using single value plus related information.

   a Eliminates some misinterpretation caused by extremely high or low results that occur rarely.

   b Gives an idea of what type of distribution is occuring (normal, skewed, etc.)

   c Average values are extremely useful but it is usually the maximum or minimum values that pose serious problems.

D Graphic Methods

One of the easiest, most attractive, and most efficient ways to present numerical information is by use of graphs.

1 Graphs commonly used

   a Normal graph paper (Cartesian Coordinate paper) - This is the most generally used graph paper and is most useful in obtaining an idea of the trend the numerical observations are taking. This type of paper is most helpful in preliminary plotting of information to decide if semi-log, log-log or other types of functional graph paper would be more desirable to use.

   b Functional graph paper (semi-log, log-log) - Use of this paper allows for the plotting of results in straight line fashion that would appear on normal graph paper (Cartesian Coordinate paper) as a curved line.

   c Arithmetic or geometric (log) probability paper - this type of graph plots the normal frequency curve (Bell shaped curve) as a straight line.

   d Nomograph - Used where complex equations or complicated mathematical manipulations and calculations are involved. Nomographs have been developed for numerous applications.

   e Special graphs

      1 3-dimensional diagrams

      2 pie-diagrams

      3 histograms, etc.

2 Desirable characteristics of graphs

   a Fairly simple

   b Be able to stand alone

   c Graphs should be designed so they may be easily reproduced.

IV SUMMARY

The presentation of information is one of the first steps in report writing. It is one of the most important parts of any report and if properly handled can produce a report that is brief, understandable and useful to the reader. The manner in which results are presented (written or oral) may serve as a basis for judgment of the whole survey.

This outline was prepared by P. F. Atkins, Jr., Sanitary Engineer, formerly with Training Activities, Ohio Basin Region, FWQA.

ORGANIZATION AND PRESENTATION OF BIOLOGICAL DATA AND REPORTS

I INTRODUCTION

A Communication between the physical and biological sciences -- significance, special problems and considerations. The disappearance of traditional boundaries that has resulted from interdisciplinary attacks on major technological problems.

B Basic requirements for clear communication: aspects of scientific writing that must be continuously emphasized and reviewed when engineers, biologists, chemists and geologists contribute to a common goal.

II THE VARIOUS FORMS THAT SCIENTIFIC COMMUNICATION TAKES

A The General and Specific Goals of Writing a Paper or Report

B Reports and Published Papers

1 Differences and similarities

2 Cautions and criteria

3 General organization

C Writing -- see Style Manual for Biological Journals. American Institute of Biological Sciences, Washington, D. C. (not discussed during this hour).

D Presentation of Data

1 Tables

a Parts of a table -- number, title, box heads, stub and field

b Superfluous data

2 Illustrations

a Types of diagrams -- point, line, bar, area, three-dimensional and pictorial

b Scales -- arithmetic, semi-logarithmic and logarithmic

c Diagrammatic representation of range, mean, variation and significant difference

d Frequency distribution, proportions, percentages and time series

e Special value of pictorial diagrams in interdisciplinary communication; example of an estuarine circulation pattern being used to interpret the natural and artificial distributions of biological populations.

3 Appendices -- their importance for present purposes is emphasized.

III THE EXPRESSION OF IDEAS AND CONCEPTS PECULIAR TO BIOLOGY AND THEIR RELEVANCE TO MARINE ENGINEERING

A Due to the indeterminate nature of many biological phenomena, concise equations are often not possible. Words, therefore, become specially important to process information and build concepts.

1 Concise terminology -- references and basic texts.

2 Fundamental simplicity of all good terminology.

B The Binomial System of Systematic Nomenclature -- Naming a Species

C Use of the Population and Community Concepts to Detect Abnormal Environmental Conditions

D Examples of Information Presented in a Manner that is Readily Understandable and Significant to a Variety of Disciplines Concerned with Coastal Waters

1 Three-dimensional diagrams of plankton composition, time and location

2 Temperature- salinity- plankton diagrams

3 Comparing community composition in time and space by graphical analysis of integral means

REFERENCES

1 Abercrombie, M., Hickman, C.J. and Johnson, M.L. A Dictionary of Biology. Penguin Books. 1957.

2 Conference of Biological Editors, Committee on form and style. Style Manual for Biological Journals. American Institute of Biological Sciences, Washington, D.C.

This manual should be at hand whenever a person sits down to write a paper or report in the biological sciences. 1972.

3 Fieser, L.F. and Fieser, M. Style Guide for Chemists. Reinhold, New York. 1960.

4 Fowler, H.W. Modern English Usage. Oxford University Press. 1926, 1958.

5 Simpson, G.G., Roe, A. and Lewontin, R.C. Quantitative Zoology Revised Edition. Harcourt, Brace, New York. Chapter 14, Graphic Methods. The various methods of graphical representation commonly used in biological publications are described. Statistical methods are covered in greater detail in other standard references, but the clarity of presentation here makes the book valuable for routine applications. 1960.

6 Strunk, W. and White, E.B. The Elements of Style. MacMillan, New York. 1959.

7 Suggestions to Authors of the Reports of the United States Geological Survey. Fifth Edition. U.S. Government Printing Office, Washington, D.C.

8 United States Government Printing Office. Style Manual. Revised Edition, January, 1959.

This outline was prepared by H.P. Jeffries, Associate Professor of Oceanography, Graduate School of Oceanography, University of Rhode Island, Kingston.

WATER QUALITY SURVEYS

PREPARATION OF SURVEY REPORTS

I TYPE OF REPORT

The type of stream survey report to be prepared depends on two basic factors. These are the purpose and the audience for whom intended.

A The Purpose of the Report

1 A report of findings or basic data

2 A report of existing causes and effects together with an explanation of how and why.

3 An exposition of existing causes and effects and a projection of conditions that reasonably may occur due to natural variations in stream flow and temperature.

4 A purpose similar to 3 above plus a prediction of the effects of population growth and industrial change.

5 The same purpose as 4 above plus an estimate of the need to protect water uses, and cause reduction in waste loads.

B The Specific Audience for Whom the Report Is Prepared

1 For the record - no expository purpose

2 Other technical agencies with competencies in the same field

3 Other technical agencies in other fields

4 Public officials supporting or opposing the recommendations of the report

5 The general public

C Both in style and content the report should be adequate to serve as a basis for action to accomplish the recommended objectives.

II ORGANIZATION OF REPORT

A Title, Authors, Contents

B Acknowledgement of Aid and Assistance

1 Can be included in a letter of transmittal or submission

2 Can be incorporated in a preface or foreword

3 Should include names of persons and of corporations, public and private, who assisted or aided the survey

C Authority

1 Source of Authority

2 Date of authorization

D Report Summary

1 A brief summary of the report and its recommendations generally precedes the report proper and should include three topics:

a Summary of specific findings

b Conclusions drawn from findings

c Recommendations in general terms.

2 Brevity is essential but not at the expense of clarity.

3 A very brief but lucid description of the stream section involved should be included.

4 This will be the only part of the report read by many of its audience. Consequently it should be drafted with the utmost care.

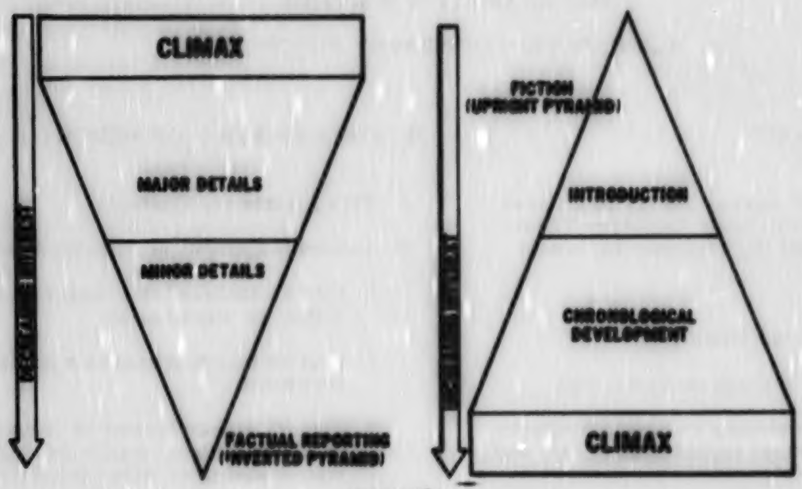

CLIMAX
MAJOR DETAILS
MINOR DETAILS
FACTUAL REPORTING
(INVERTED PYRAMID)
FICTION
(UPRIGHT PYRAMID)
INTRODUCTION
CHRONOLOGICAL
DEVELOPMENT
CLIMAX

Figure 1

5 Figure 1 illustrates the principles of written communication format. It is important that technical reports be presented in factual report arrangement and NOT in one similar to that of fiction.

6 Figure 2 shows how the various portions of a survey report relate to the generalized factual report.

7 Recommendations, although briefly stated in general terms, should be couched in positive, unexaggerated language.

8 Cost estimates of compliance with recommendations is helpful but not essential.

9 Both tangible and intangible benefits may be listed briefly under conclusions.

E INTRODUCTION

The body of the report should begin with a statement of the problem and a discussion of the reasons for and the location of the study.

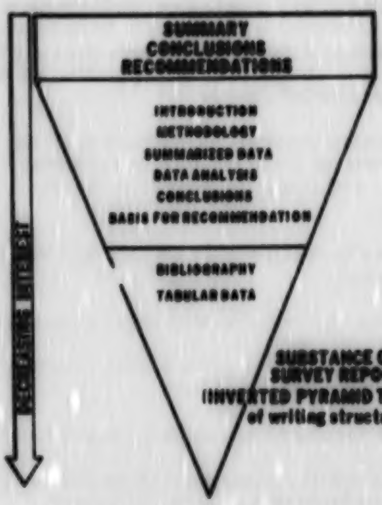

SUMMARY
CONCLUSIONS
RECOMMENDATIONS
INTRODUCTION
METHODOLOGY
SUMMARIZED DATA
DATA ANALYSIS
CONCLUSIONS
BASIS FOR RECOMMENDATION
BIBLIOGRAPHY
TABULAR DATA
SUBSTANCE OF
SURVEY REPORT
(INVERTED PYRAMID TYPE)
of writing structure

Figure 2

1 Statement of the problem

a A description of the area, emphasizing pertinent features, should be included.

1) The inclusion of pertinent historical data is of value for audience orientation.

2) The relationship of this study to other current water resource studies.

3) Water use and economic data may be important.

b An area map is an absolute necessity.

1) Features included should be carefully selected and section of the stream involved should be emphasized.

2) Do not include unnecessary detail.

3) A general location map usually orients the reader to the area map.

2 Objectives of the Survey

a A statement of the purpose by listing the specific answers sought by the survey to address various aspects of the problem.

b The geographical and time scope of the objectives of the survey.

F Survey Methodology

A complete description of the methods of study employed is an important part of the record.

1 The time period of survey should be noted.

2 All sampling and gauging station locations should be identified by river mile.

3 Sampling and analytical methods

a Provide adequate description of all non-standard methods.

b An appendix for these descriptions may be required if they are lengthy.

4 Frequency of sampling

5 Description of laboratory types and locations

6 Hydrological methods employed for:

a Times of water travel

b Stream flow data

c Any waste flow measurements

G Survey Results

1 Sources of wastes

a Computed waste loads based on known contributing populations and industrial waste strength

b Results of sampling and gauging program

c Data summaries or displays suffice for text of report.

2 Stream data

a Summarized survey in the text

b Complete tabulations including time of collection and averages in appendix

3 Hydrological data

a Usually tabulated with analytical results both in the text and in appendices

b Time of water travel curve or curves

c Stream flow frequency charts

d Pertinent groundwater data

4 Aesthetic considerations are of real importance.

H Analysis and Interpretation of Data

1 Three fundamental procedures are required:

a Comparison of survey results and appropriate water quality criteria

b Projection of survey data to provide for comparison of stream conditions with water quality criteria under more adverse conditions

c Estimates of permissible wastes loads under present and future conditions.

2 Since this topic is the subject of other outlines it will not be further developed here.

3 A description of the methods of analysis and interpretation belong in the report.

a Necessary assumptions should be stated.

b All statistical methods used should be identified.

4 Results of analysis and interpretation are best presented in chart form insofar as possible, to support discussion and interpretation rationale.

I Conclusions

1 Focus of attention, much of it critical, is on this section of the report.

2 Clearness, conciseness, and positiveness are essential.

3 This section indicates reasoning that leads from findings to conclusions.

J Recommendations

1 This is the crux of the report and should answer the question, "What needs to be done to resolve problems delineated in the report?"

2 Cost estimates are highly desirable, if possible, and appropriate.

K Bibliography

1 Useful to the student and perhaps to future workers

2 Entails additional time and effort in assembling references in proper form

III TECHNIQUES AND TOOLS

A Prepare a Detailed Outline

1 This is an important step if the coverage of the report is to be complete and its arrangement logical. It is also a time-saver if properly done.

2 Topics to be included in the outline will become apparent from the foregoing discussion of report content.

B Words are Your Tools

1 Keep dictionary, thesaurus, and glossary available.

2 Define any vague terms or abbreviations.

3 Control superlatives and slang.

4 Avoid emphatics such as "it is to be noted" or "it is a well-known fact".

5 Limit intensive expressions, such as "extremely" or "undoubtedly."

6 Use active verbs when possible.

C Regularly review the report organization and development with a colleague.

This outline was prepared by Staff Members, National Training Center, WPO, EPA, Cincinnati, OH 45268.

AN INITIATION INTO STATISTICS

I INTRODUCTION

Quantitative statistical analysis has been publicized by some analyst as a cure all. This exaggerated claim is adhered to by only a few opportunists.

Quantitative analysis should be thought of as a valuable tool which can be exploited by close cooperation between the statistician and the investigator in a joint attempt to find better methods of controlling or understanding the complex interaction of men, materials, machines and the natural environmental resources.

Modern statistics is still a relatively new subject with along list of unsolved statistical problems. Unless caution is observed the inevitable result will be some unhappy experiences with inadequately equipped amateurs. In point of fact, most statistical procedures are quite simple and intuitively acceptable once they have been pointed out in a particular context. Heuristic persuasion can be effectively used to give insights into the concept, development and foundations of the particular tool used.

II IMPORTANCE OF PREPLANNING

Many investigators now believe that statistics and particularly the statistical design of data collection has potentially impressive leverage effects on the amount of information to be included and obtainable from any research effort.

After all, any finite body of data contains only a limited amount of information. This limit is set by the inherent nature of the data themselves and cannot be increased by any amount of ingenuity exercised by the data analyst. However intelligent preplanning can increase this limit.

Statistical methods cannot reveal anything that is not already implicit in the data. However, every correct inference of interest possible should be made. A continual effort should be made to recover all of the information but there never will be a way to find more than is already there.

It would be better to do enough work on five projects than an inadequate amount on a much larger number. You don't have to plan in order to fail, all you have to do is fail to plan.

Many investigators still believe that statisticians only enter at the end of the work--that is, at the final examination of the data. It is small comfort to the investigator to be told after the event, that the answers to his questions are inconclusive and were bound to be so because an inadequate amount of effort was allotted to the work.

It is well known that the end doesn't justify the means but in data collection and analysis the end dictates the means. The importance of preplanning cannot be overemphasized. If you fail to plan then you are planning to fail.

Any investigation problem can be viewed as a five step process. These are:

1 Problem definition

2 Data collection scheme

3 Actual data collection

4 Data analysis

5 Written report or recommendations

The most important step is the first one because the second is based upon it. The third follows from the second, etc. The process is like a chain with five links. If the first link is incorrect, the entire chain is. Too often, too little time is spent on step one. Many times the problem is vaguely or ill defined. The best protection against these faults is to write down black on white a statement of the problem and have all connected with the investigation to agree on the statement. With this as an introduction we proceed to describe the field of statistics briefly.

III ALTERNATIVE VIEWS OF STATISTICS

The field of statistic can be described from many different angles. Each viewpoint results in a different insight into this vast discipline. In this introduction we consider the entire field from three viewpoints. The first view is very simple, the second introduces more details and finally the last viewpoint is very comprehensive with respect to subject matter but is only one molecule deep.

IV FIRST VIEWPOINT

The first way of looking at statistics is to partition the data source into the method of acquisition as either experimental or nonexperimental.

FIGURE 1

FOURFOLD MAP OF ENDS AND MEANS IN APPLIED STATISTICS

PURPOSE OR ENDS OF STATISTICS		Source Or Means Of Data Acquisition	
		Nonexperimental	Experimental
Enumerative or Descriptive Answers: question, how much	Aim: adjustment to the environment	Tables and graphs Averages central tendency Dispersion measures Frequency curves Correlation Survey sampling Index numbers	) Same Assessment of probabilities Theoretical means, variances,etc. and corresponding confidence intervals
Explanatory or analytic causal inference Answers: question, why	Aim: ascendancy over the environment	Specialized branches of applied statistics Demography Econometrics Genetics Causal inference from time series	Design of factorial experiments Randomization methods Significance tests Regression analysis Analysis of variance and covariance Probit analysis

In an experimental investigation, control is exercised over some or all of the influencing variables, while in a nonexperimental investigation nature is merely measured as is without any control on any of the influencing variables.

The purpose of the analysis is also viewed as two-fold, that is, either enumerative or analytic. The combination of double means and ends leads to the four-fold table in Figure 1. This is taken essentially from reference 1.

Historically, the field of statistics started with the upper left quadrant about the turn of the century. The research proceeded clockwise until the lower left hand quadrant was reached. That is causal inference from observational data. This quadrant is the most difficult and has been receiving the most research attention lately.

In each quadrant some of the techniques are indicated and the reader should be further cautioned that the lines drawn are fluid and are not rigid. A partial listing has been given in each quadrant and no attempt has been made to give a 100% enumeration.

V SECOND VIEWPOINT

The next viewpoint of statistics is in this orientation the one taken by The International Statistical Institute. Figure 2 shows their scheme for classification of abstracts. Any article in statistics can be classified in one or more of the indicated titles. Some articles are given a primary classification and others in addition are given a secondary classification.

Let us give a brief introduction into the major topics listed in Figure 2.

Upon inspection of the subtitles of the first topic, mathematical methods, it is evident that these subtitles are a list of the mathematical techniques used by the professionals to solve the difficult problems encountered in the development of the theory and methods.

FIGURE 2

SCHEME FOR CLASSIFICATION OF ABSTRACTS

0. MATHEMATICAL METHODS (White)
    0. General papers
    1. Solution of equations
    2. Methods of curve and surface fitting; smoothing
    3. Interpolation and quadrature
    4. Special functions and transforms
    5. Functional relationships
    6. Determinantal and matrix analysis
    7. Game theory
    8. Programming techniques
    9. Group and field theory
    10. Graph theory and combinatorial analysis
    11. Measure theory
    12. Optimisation

1. PROBABILITY (Pink)
    0. General papers
    1. Calculus of probabilities
    2. Expected values
    3. Combinatorial problems
    4. Geometric probability
    5. Limit theorems
    6. Stochastic convergence
    7. Stochastic approximation
    8. Decision theory and functions
    9. Transforms: Fourier, Laplace, etc.
    10. Convolutions

2. FREQUENCY DISTRIBUTIONS (Green)
    0. General papers
    1. Descriptive properties
    2. Transformations of variates
    3. Normal and lognormal
    4. Binomial, multinomial and hypergeometric
    5. Poisson, exponential, negative binomial, logarithmic and contagious
    6. Rectangular, extreme value and Weibull
    7. Pearson and "series expansion" distributions
    8. Truncated and mixed distributions
    9. Multivariate distributions
    10. Limit distributions
    11. Approximations
    12. Other distributions

FIGURE 2 (continued)

3. SAMPLING DISTRIBUTIONS (Light Blue)

0. General papers
1. t, z, F and x^2 distributions
2. Non-central distributions
3. Studentisation
4. Quadratic forms
5. Correlation and regression coefficients
6. Location and scale statistics
7. Shape and other descriptive statistics
8. Order statistics
9. Multivariate problems
10. Limit distributions
11. Linear forms

4. ESTIMATION (Yellow)

0. General papers
1. Properties of estimators
2. Types of estimator: Bayes, maximum likelihood, least squares, etc.
3. Individual estimators: point
4. Individual estimators: interval
5. Inequalities: tolerance limits and regions
6. Distribution-free methods
7. Sequential methods
8. Multivariate problems
9. Finite population procedures - surveys
10. Simultaneous estimation
11. Distribution functions and densities
12. Decision theory

5. HYPOTHESIS TESTING (Purple)

0. General papers
1. Properties of test
2. Individual hypotheses
3. Two-sample problem
4. k-sample problem
5. Outliers
6. Distribution-free tests
7. Sequential tests
8. Multivariate problems
9. Types of test: likelihood ratio, Bayes, minimax, etc.
10. Goodness-of-fit tests
11. Combining and comparing tests
12. Decision theory

6. RELATIONSHIPS (Grey)

0. General papers
1. Regression; linear hypothesis, polynomials
2. Correlation inc. canonical correlation
3. Factor methods and principal components
4. Discriminant analysis and cluster analysis
5. Ranking and scaling methods
6. Systems of equations: structure
7. Non-linear equations-logistic
8. Transformed relationships-quantal response
9. Association and contingency
10. Functional relationships
11. Non-standard conditions
12. Other multivariate methods

7. VARIANCE ANALYSIS (Biscuit)

0. General papers
1. Fixed effects model
2. Variance components model
3. Mixed and other models
4. Non-orthogonal data and missing values
5. Non-standard conditions-failure of assumptions
6. Covariance analysis
7. Multiple comparisons; multiple decision procedures
8. Ranked data
9. Sequential methods inc. preliminary tests
10. Combining sets of results
11. Precision of measurement
12. Multivariate models

8. SAMPLING DESIGN (Orange)

0. General papers
1. Simple random; stratified; multi-stage
2. Sampling with unequal probability
3. Multi-phase sampling; double sampling
4. Natural (human, animal and biological) populations
5. Non-sampling problems
6. Censored, systematic and quota sampling
7. Nature and number of units; cost and efficiency
8. Acceptance inspection
9. Process control

FIGURE 2 (continued)

9. DESIGN OF EXPERIMENTS (Blue)

0. General papers
1. Block designs; designs for two-way elimination of heterogeneity
2. Factorial arrangements
3. Response surfaces
4. Nature of unit; number of replications; cost and efficiency
5. Paired comparisons and matching problems
6. Preference tests
7. Repeated and sequential experiments
8. Weighing problems
9. Sensitivity problems
10. Systematic designs
11. Screening tests
12. Other designs, e.g., mixtures

10. STOCHASTIC PROCESSES AND TIME SERIES (Red)

0. General papers
1. Properties of individual process
2. Estimation problems
3. Tests of hypotheses
4. Queueing, storage, risk and congestion theory
5. Information theory
6. Stationary processes and spectral analysis
7. Auto and serial correlation
8. Multivariate processes
9. Biological population studies; genetic models
10. Renewal theory
11. Markov processes
12. Branching processes

11. MISCELLANEOUS AND SPECIAL TOPICS (Cream)

0. General statistical methodology
1. Statistical tables and charts
2. Probability graph papers
3. Nomograms and graphic methods
4. Machine methods; hand and punched cards
5. Machine methods; electronic digital
6. Machine methods; other
7. Monte Carlo methods
8. Index numbers
9. History, biography and bibliography
10. Inventory
11. Life-testing and reliability
12. Teaching and training methods

These are the tools used to extend the present boundaries of knowledge. They are not statistics per se but are an indispensable aid to the research statistician.

The next topic, probability, can be described as a method for quantitizing uncertainty where the limits of uncertainty are a numerical value which does not lie outside the range from zero to one. Most people have a correct intuitive feel for probability. If an event can happen (i. e., it will rain) say with probability 0.9 (or 90%) then one knows that the event could or could not occur. However, for a large number of trials, on the average nine times out of ten it will occur (or rain) and one in ten it won't.

There is no universally acceptable abstract calculus of probability. That is to say if one adopted a set of axioms and developed the theory of probability then the inevitable result would be some controversy and many heated arguments. Some fault would be found in the development of the theory by the purists. The purists are those who are concerned with the philosophical and correct logical foundations. They are necessary and serve a useful purpose. They are not boat rockers but keep it from sinking because they plug any holes that may be present.

Most statisticians view probability from a practical viewpoint and their attitude is that it works and does a good job, so let the purists thrash out the difficulties.

In the third topic, frequency distribution, studies are concerned with characterizing and identifying the distribution of a variable or measurable characteristic. The most famous of these distribution is the bell shaped symmetric curve known as the normal distribution. It is characterized by two parameters, namely, the mean, a measure of central tendency and the standard deviation, a measure of spread or variability.

The list of distribution used in statistic is greater than the subtitles listed. This list includes only the most important ones and a complete list of distributions developed by researchers with their properties would fill a book.

The usefulness of a frequency distribution lies in the fact that from it one can calculate the probability of occurrence of different values or set of values for the variable.

Very few people have the luxury when doing research of measuring every unit in the population. For this reason sampling distribution per se are studied quite vigorously. Constraints of time, money, people and resources needed, versus available, force researchers into taking a sample or a subset of the entire population. On the basis of a sample, decision statements or estimates are made about the entire population. Different samples have different values or a sample varies from one sample set to another. Each sample as well as any value calculated from it has its own sampling distribution. These must be known before quantitative statements can be made.

The three most important sampling distributions are the t, F, and Chi-square. They, as well as the normal, are tabulated in almost every textbook on statistics.

In the fifth subject, estimation, the sample values are used to infer information about the entire population. For example, from a sample, one can calculate the sample mean and use this to make statements about the parent population mean. The calculated sample mean ($\bar{X}$) is an estimate of the population mean (the symbol used is the Greek letter mu).

One legitimate question that can be asked is "Is this the best possible way?" Best possible way is a fuzzy concept and must be unfuzzied before the question can be answered. That procedure produces many interesting paths as evidenced by the list of subtitles.

Hypothesis testing (or significance testing), our next topic, is a technique that is too often not really understood. It can be understood by anyone since the concept is really quite simple and is analogous to a comparison procedure familiar to everyone. A test of hypothesis is the familiar method of comparing two things (an unknown and a known) for identity or nonidentity. After the comparison has been completed then one of two conclusions is made: 1) the two things compared are not identical or 2) the things compared are identical.

In the world of statistics, the unknown in the comparison procedure is the sampled population. The known used for the comparison is a hypothetical population which is also called the mathematical model or the test assumptions.

The sampled population values are used to evaluate a statistic which can be thought of as a formula for combining the information available in the sample. The observed statistic value obtained from the sample is compared with corresponding values obtained from the mathematical model.

If the observed and expected statistic values are in disagreement then the sampled and hypothetical population disagree or are not identical. If the statistic values agree the populations agree or are identical.

A simple example will make the ideas clear. Assume you are sampling from normal with an unknown mean and a standard deviation equal to ten. We wish to test if the unknown mean equal five. The mathematical model or known is normal, mean = 5 and standard deviation = 10. If the conclusion of the comparison procedure is agreement between the sampled population and the mathematical model. This means both normals have equal means and standard deviations. That is equivalent to saying the two means are equal to five or the unknown mean from the sampled population equals five. If the statistical decision was the two populations disagree then the evidence says that the mean of the sampled population doesn't equal five.

In summary, a one sentence nontechnical definition for a test of hypothesis could be: "A test of hypothesis can be defined as a method for comparing an unknown sampled population with a known population (or mathematical model) and deciding if the two populations are in agreement (or are alike) or disagreement (or are not alike)."

A longer nontechnical working definition for hypothesis testing would be as follows. A test of significance can be defined as a method of analyzing data so as to discriminate between two hypotheses. The first hypothesis is called the null hypothesis which means there is no difference between sampled and hypothetical population. The difference between the two populations is equal to zero or is null. The second hypothesis is called the alternative hypothesis which should be the operational statement of the experimenters research hypothesis. Next the best test for the alternative hypothesis is selected, that is, the one that has the highest probability of saying the research hypothesis is true when it is in fact true. The probability of deciding the null hypothesis is false when true is selected by the researcher. This value is called the alpha-error. The alpha-error specifies those values for the statistic for which the decision is agreement (or do not reject H_o or there is no difference between sampled and hypothetical populations) or there is disagreement (accept alternative or research hypothesis). The sample is now calculated and the statistic value is compared with the decisions values determined by the alpha-error. The decision is made to either: 1) do not reject the null hypothesis or, 2) reject the null hypothesis (or equivalently accept research hypothesis).

For those readers who want an in depth nontechnical explanation of a tests of significance, the references listed by this writer are suggested as a beginning.

Our next topic relationship needs no introduction since every scientist understands that some sort of predictive technique is implied.

Variance analysis is next on our list. In variance analysis, the total variation displayed by a set of observations may, in certain circumstances be separated into components associated with defined sources of variation. The defined sources are used as a criterion of classification for the observations. That portion of variance which is left undefined or explained is called experimental error. Many standard situations can be reduced to the variance analysis form.

Our ninth topic has many aliases, two examples are sampling plan and survey design. The purpose is to measure nature as it exists by taking a sample from a population. Statistical data are collected to provide a rational basis for action. The action may call for the enumerative interpretation of the data or it may call for an analytic interpretation. These actions determine the difference between enumerative and analytic surveys. Enumerative surveys would be classified in the upper left hand quadrant of Figure 1, while analytic surveys should appear in the lower left quadrant of that same figure.

In a design of experiment, our next topic, the researcher is interested in the change produced in a response by the influencing factors. For example, a researcher may be interested in the yield in bushels of wheat (the response) when different fertilizers are used in different amounts (the influencing factors under his control). The design of experiment consists of the set of rules which match treatments (or list of influencing factors to be studied) with the units (plots of ground) to which the treatments are to be applied. The principles of randomization, replication and stratification are employed and produce the many designs available.

In the eleventh topics, the adjective stochastic implies the presence of a random variable. Hence a stochastic process is one wherein the system incorporates an element of randomness as opposed to a deterministic system. A time series is a set of ordered observations on a measurable characteristic taken at different points in time.

The final classification is the miscellaneous basket for topics which are not large enough for their own major classification.

VI THIRD VIEWPOINT

The final viewpoint will be the question and answer approach. This will lead to a more detailed problem classification viewpoint and tie in some of the topics discussed in the second viewpoint.

Suppose you are interested in the B.O.D. measurements at a particular point in the Bay of San Francisco. The totality of all such measurements will be called a parent population and will be represented by some distribution as shown in Figure 3.

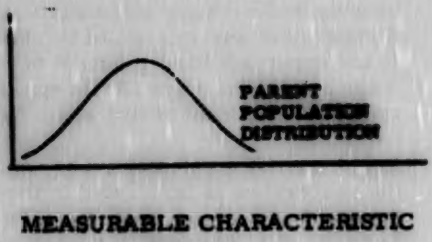


Figure 3

Three questions the researcher can ask at this point are:

1) What is the research hypothesis? Does my boss agree?

2) What is the definition of our population?

3) Am I going to study my population as is or subdivide it and study each subdivision?

Let us assume that the answer to the first question in our imaginary study has been finalized. Question one is still the best starting point in any investigation.

In this imaginary study, the answer to the second question is clearly answered but in other studies, the question is not always easily answered.

Some answers to the third question could be as follows: I will subdivide the population and compare daytime versus nighttime. An alternative choice could be, I will subdivide into the four seasons and compare.

After these three questions have been satisfactorily answered, the next question could be:

4) Will I be able to measure the entire population or take a sample?

If the answer to this question is: I will measure all the population, then the data analysis will be descriptive statistics. Statistical analysis is subdivided into the two main areas, namely descriptive and inferential statistics.

The first of these is descriptive statistics which is concerned with the problem of how you condense a large mass of data in such a way so that you summarize the available data into several succinct numbers which is simple to interpret and easier for the mind to grasp and comprehend. This type of statistic is the one students first meet in a course in statistics (See Figure 4).

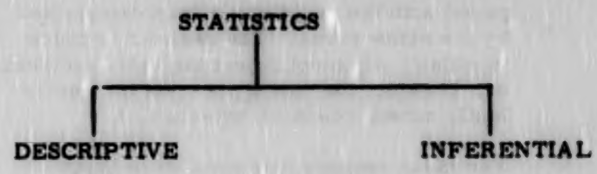


FIGURE 4

If the answer to question 4 is take a sample (few investigators have the luxury of measuring the entire population) then several other good questions occur like the following:

5) How big a sample do I need? Is my budget adequate for it?

6) If sample size is not fixed in advance, how will data collection be terminated?

7) How will our samples be obtained? Time, place, order, etc.

8) If our samples are not random, how will we generalize?

Statistical theory assumes random samples, hence violation of this assumption invalidates the application of the theory.

9) Am I going to use the sample for statistical inference with respect to estimation or hypothesis testing? (See Figures 5 and 6).

Hypothesis testing can be briefly defined as a method of analyzing data so as to discriminate between two hypotheses. The one hypothesis is the research hypothesis while the other is generally the negation of the research hypothesis.

Estimation is concerned with statements about the numerical value of unknown population parameters from sample data. If the estimate is a single number, then it is called a point estimate. If an interval is calculated within which the parameter lies in a probability sense, then it is called an interval estimate. (See Figure 7)

Hypothesis testing can be subdivided into two broad types.

If the parent population is normally distributed, then the hypothesis testing is said to be parametric. If normality cannot be proven or safely assumed, then the test is said to be nonparametric. (See Figure 7)

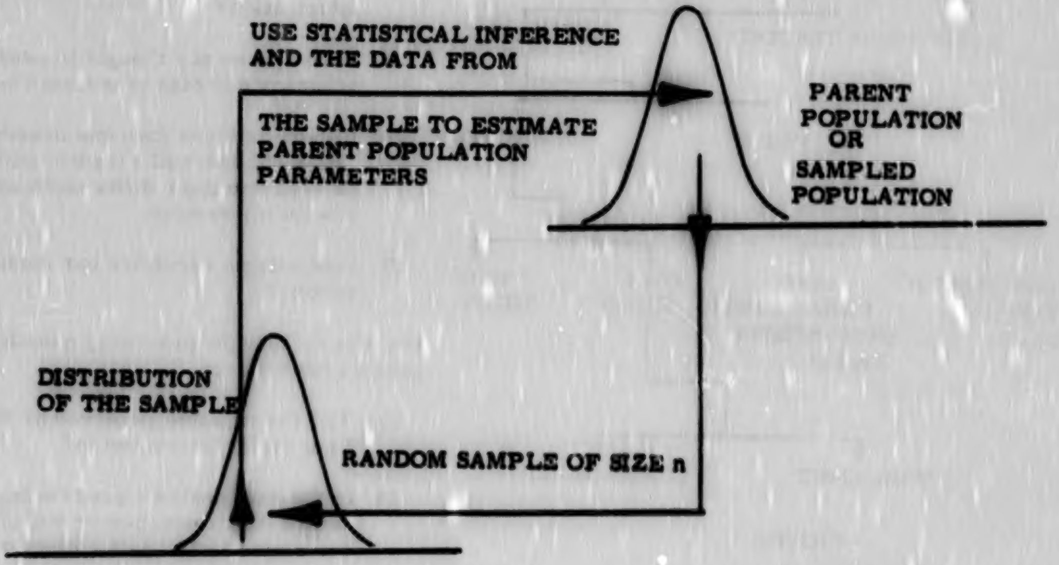

USE STATISTICAL INFERENCE AND THE DATA FROM
THE SAMPLE TO ESTIMATE PARENT POPULATION PARAMETERS
PARENT POPULATION OR SAMPLED POPULATION
DISTRIBUTION OF THE SAMPLE
RANDOM SAMPLE OF SIZE n

FIGURE 5

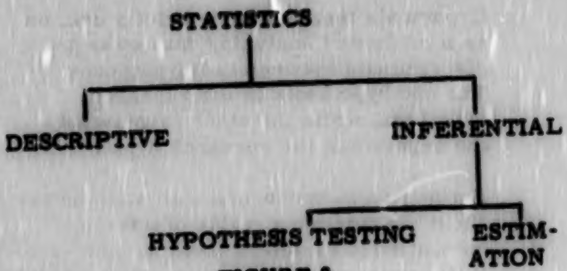

STATISTICS
DESCRIPTIVE
INFERENTIAL
HYPOTHESIS TESTING
ESTIM-ATION

FIGURE 6

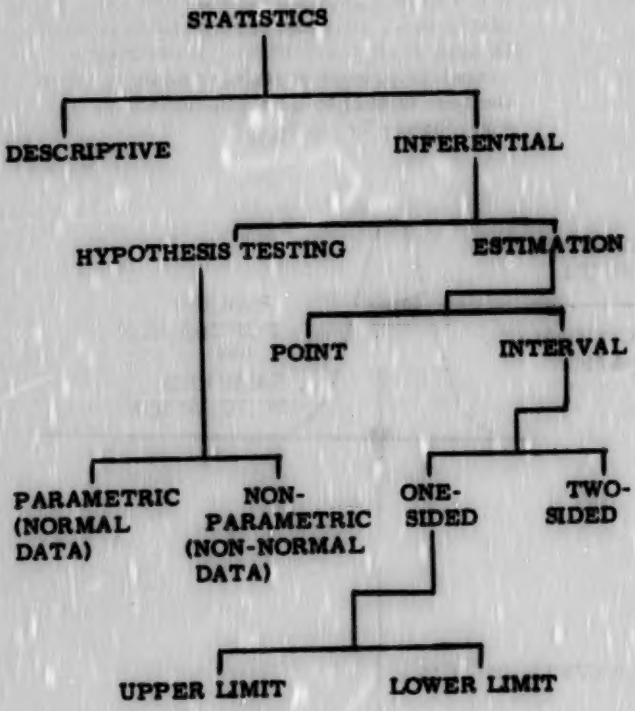

STATISTICS
DESCRIPTIVE
INFERENTIAL
HYPOTHESIS TESTING
ESTIMATION
POINT
INTERVAL
PARAMETRIC (NORMAL DATA)
NON-PARAMETRIC (NON-NORMAL DATA)
ONE-SIDED
TWO-SIDED
UPPER LIMIT
LOWER LIMIT

FIGURE 7

If hypothesis testing is to be applied, then some pertinent questions are:

10) What is the null hypothesis?

11) What is the alternative hypothesis?

12) What is the Type I error?

13) What is the Type II error?

14) What is the consequence of a Type I error?

15) What is the consequence of a Type II error?

At this point, other thought provoking questions can be asked.

16) What are the variables not measured that affect my observations?

17) Are we going to reject observations that seem aberrant? If so, on what basis, objective or subjective?

18) Have I given any thought to lost information?

19) Have I given any thought to precision, accuracy and bias of measurements?

20) If there is more than one observer involved, how will I insure uniformity or separate their differences as it affects my results.

21) How will we minimize our nonsampling errors?

Some more thought-provoking questions of general nature are:

22) Will the variable observed be the one I am really interested in?

23) Is the data analysis predetermined before data collection or will this be determined after data collection?

24) What is it we are trying to do?

25) What do I have to work with?

26) What do I need to know?

27) Who else is concerned or should be?

I would like to close this section by asking some pointed questions related to the computer.

28) Is a computer going to be used or will the analyses be manual?

29) If a computer is going to be used, are the programs needed available?

30) If the computer programs are not available now, will they be ready when the data has been collected?

31) If the computer is going to be used and a large mass of data will have to be entered, how will the data be fed into the computer?

VII RECOMMENDATIONS

There are many short courses in statistics available through EPA National Training Center. Figures 8 and 9 list the 5-day courses and the 8-hour seminars dealing with statistics.

FIGURE 8

5-DAY	
Number	Title
801	Basic Environmental Statistics
	Environmental Statistics
802	Design of Experiments
804	Nonparametric
806	Analyzing Qualitative Data
810	Applied Regression Analysis
815	Sample Size Determination
820	Survey Sampling

FIGURE 9

8-Hour Seminars	
Number	Title
899.1	Survey Sampling for Managers
899.2	Analysis of Variance and Design of Experiments
899.3	Regression and Correlation
899.4	An Introduction to Hypothesis Testing
899.5	Statistical Quality Control
899.6	Estimation and Hypothesis Testing for Normal
899.7	Nonparametric Hypothesis Testing
899.8	Introduction to and the Analysis of Categorical Data
899.9	An Introduction to Probability

The five-day courses are concerned with applications while the seminars are designed to acquaint the listeners with the topics and the type problems that can be solved.

REFERENCES

1 Wold, H. Causal Inference from Observational Data. J. R. Statist. Soc. A. 119. 28. 1956.

2 Santner, J. F. An Introduction to Tests of Significance Training Manual, National Training Center, DTTB, MDS, OWP, EPA.

3 Santner, J. F. Variations on a Test of Significance Training Manual, National Training Center, DTTB, MDS, OWP, EPA.

This outline was prepared by Mr. J. F. Santner, Mathematical Statistician, National Training Center, MDS, OWP, EPA, Cincinnati, OH 45268.

ADMINISTRATIVE NEED FOR BIOLOGICAL INFORMATION

Water pollution control supervisors are faced with especially difficult administrative problems. So vast are their responsibilities that they are forced to rely upon information derived from a number of professional disciplines. And more will be expected of such supervisors in the future. To contribute most effectively to a program each chemist, biologist, etc. must understand the needs of the program. He needs to recognize where he fits into the system in order to plan his work for maximum benefits.

Often times there seems to be a disparity between the information required by a supervisor and that submitted by his staff. There is a simple way to correct this situation, i.e., base all work on specific administrative need. This outline attempts to categorize these requirements and present a few suggestions whereby biologists in particular may insure maximum benefit from work performed.

I RESPONSIBILITIES OF AN ADMINISTRATOR

Categorization of the duties of an administrator is a difficult and nebulous task at best. However, six primary responsibilities are evident. An administrator must:

A Formulate Programs

This responsibility generally requires rapid assessment of an existing situation followed by formulation of a corrective program by selection from a number of alternatives. The alternatives may be proposed by the administrator, his staff, or both.

1 Since information required for problem definition usually must be rapidly-collected and comprehensive, it is commonly based on key concepts. For instance, a chemist charged with preliminary determination of water quality might perform only key analyses. Likewise, a biologist might examine key groups of organisms or associations. Such procedures are rapid and yet provide information which constitutes a basis for further action.

2 Efficient administration requires effective management of four commodities: money, manpower, mission, and material. To the extent which actions of staff aid administrators in effective management such actions are beneficial. Initially, administrators are concerned with the contribution which a proposal can make toward solution of the defined problem. To a subordinate proposing a plan this should be a paramount consideration. Manpower, financial, and equipment adjustments come later.

3 Despite the technical limitations of the term, "indicator organism", biological data are often used in exactly this manner. However, administrators must understand that the presence or absence of a given organism is not confirmatory evidence of pollution. The indicator organism occupies much the same position as the presumptive test in bacteriology; its presence or absence may not confirm pollution but does serve as a basis for further action.

B Initiate Programs

All administrators have superiors and accordingly are expected to convince superiors that proposed programs are necessary. This means that any information supplied during program planning phases must be not only comprehensive but also convincing. The manner in which data is reported can make the difference between enthusiastic acceptance and discouraging rejection.

C Guide Program Development

All administrators like to see results - results which indicate progress in the right direction at an optimum rate. When supplying interim reports contributors must demonstrate three attributes of work performed:

1 Quality - administrators must know what was done. It helps to divide accomplishment into four or five categories during description.

2 Quantity - administrators must know how much was done. A simple statement of the percentage of work completed or further time required for completion is impressive.

3 Pertinence - administrators must be convinced that work performed is compatible with the overall objective. A simple statement that there were 25,000 sludge worms per square foot present at Station A is useless and, even worse, possibly misleading, to the administrator who does not know that only one sample was taken or that lack of equipment forced use of an Ekman rather than a Petersen dredge.

The requirement for interpretation is truly a golden opportunity for a subordinate, for no supervisor, no matter how competent, can fully appreciate all the technical ramifications of work performed by his staff.

D Formulate Conclusions

All administrators must formulate conclusions, whether they be recommendations or statements of fact. Normally conclusions are based on interim conclusions or recommendations supplied by staff. Staff members are obligated to make their conclusions (or recommendations) clear and definitive.

Much has been said about biologists' reluctance to make conclusions. Generally, this impression is not supported by facts; biologists will and do make conclusions within the limit of technical support. It must be understood that biological information simply describes the resultant effect of many variables which affect life. Only rarely does it indicate the degree of predominance, or even the presence of, any single variable.

E Support Conclusions

Each time an administrator makes a recommendation or publishes a conclusion he lays his professional reputation "on the line." Therefore, staff in all disciplines are obligated to make certain that their interim conclusions or recommendations are supported by the best possible evidence. The credibility of an otherwise fine report can be devastated by an unsupported generalization in a specialized area.

Biological data offers a special advantage for data support. Of all the types of data used in water pollution control work it alone is associated directly with life. Administrators generally demonstrate six concepts with biological data:

1 Life or death

There is hardly any more convincing information than proof that a pollutional condition or polluting substance has caused death. On the other hand, an abundance of life can be extremely encouraging.

2 Distribution

Allegations of pollution generally require proof that some change has taken or is taking place. Administrators often demonstrate these changes with tables showing presence or absence of organisms or groups of organisms at varying times and locations.

3 Toxicity and physiological effects

Data indicating toxicity and physiological effect carry the same import as do death and injury statistics for human beings. These data can often be associated directly with specific deleterious substances.

4 Productivity

Although species lists, etc. indicate distribution, they do not indicate the degree of predominance of a group or species at a given place or time. Only numerical enumerations provide this capacity.

5 Ecological relationship

Biologists have found that each creature has its own natural function. Predatory organisms prevent overpopulation in other species; some mosses, etc. provide shelter for small beetles, etc. Alteration of a natural ecological relationship may have economic, aesthetic, and other effects.

6 Non-ecological relationship

Certain physical and chemical characteristics of biota may have economic, aesthetic, or other significance. For instance, certain diatoms tend to clog rapid sand filters or may impart disagreeable taste to finished water. In this area in particular the administrator is forced to rely upon the advice of the professional biologist.

F Satisfy Superiors and/or the Public

Presentation of a report to his superiors can be the administrator's moment of truth. Presentation of a report to the public can be an even more harrowing experience. Not only must data then be meaningful and comprehensive, but also it must be presented in an easily assimilated form. The subordinate biologist may need to alter the form of presentation of his data to meet this need.

II SUMMARY

This outline categorizes the duties of a water pollution administrator. The subordinate biologist may need to alter both the type of data and form of its presentation to suit the specific administrative task required. Generally, all data are characterized by qualitative and quantitative information and may require considerable interpretation. Because of its psychological association with life biological data is uniquely impressive.

This outline was prepared by F.P. Nixon, Former Acting Regional Training Officer, Northeast Regional Training Center, EPA, Edison Water Quality Laboratory, Edison, NJ 08817.

DISSEMINATION OF TECHNICAL INFORMATION TO THE PUBLIC

I WHAT IS COMMUNICATION?

A Communication is the transference of an idea from the giver's mind (the communicator - speaker/writer) to the receiver's mind (the listener/reader/viewer).

B This transfer of ideas is accomplished through the use of symbols (words/pictures).

II EFFECTIVE COMMUNICATION REQUIRES A CHANGE OF ATTITUDE ON THE PART OF THE COMMUNICATOR.

This behavioral change is necessary because of the almost universally unrealistic approach of the nonprofessional communicator.

A The change begins with facing the fact of responsibility. The communicator is responsible for the effective transference of his ideas, not the receiver.

B The change requires a realistic approach.

What is a realistic approach?

1 To see yourself in the role of the receiver instead of the giver.

2 To understand that not what is important is talked about, but what is talked about becomes important.

3 To forget what the listener ought to do, and find out what he does do, how he reacts.

4 To define realistically (this means honestly) the purpose (end result desired) of your communication:

Inform for understanding,

Inform for understanding and action,

Please the boss,

Because Washington told you to write something,

Please your vanity.

III WHAT MUST YOU DO TO ACCOMPLISH EFFECTIVE COMMUNICATION?

A You must get your listener's attention.

B You must hold his attention.

C You must be understood, not in the sense that one understands a language (English, German, Polish, French, etc.), but in the sense of getting into your receiver's consciousness, his awareness.

D Here are proved techniques for accomplishing ABC above.

1 Evaluate your audience.

2 Use the form and style of your medium and try to understand why that medium, especially a newspaper, uses its particular style.

3 Do not open with a cliche. (There is a pressing need...).

4 Use picture words (concrete versus abstract terms).

5 Talk into the receiver's experience. Tell the unknown in terms of the known. Use words (symbols) with which the receiver can identify himself.

6 Forget about "levels" of intelligence. Do not fear that you are debasing the status of your profession, or your position, to talk into the public's understanding. And do not underestimate the value of the public's understanding.

7 Never confuse talking into the receiver's experience with cheapness and demagoguery.

8 Evaluate the results of your communication. Reliable measurements are available.

And remember, barring unavoidable contingencies such as front-page murders, a stock market crash, or war, failure to communicate is not the failure of the paper, or of the reader, but of the communicator.

Seek and do not resent criticism. Accept what is valid and forget what is not. And DO NOT be upset by the adverse reaction of the few, when you are communicating to the public.

IV WHAT THE REALISTIC APPROACH IS NOT

It is not an idealistic, emotional, impossible approach, but a tested, proved, black-side-of-the-ledger technique.

V THE COMMUNICATION OF TECHNICAL INFORMATION TO THE PUBLIC IS IMPORTANT.

Consider what the world might be, and rapidly become, if people with important technical information really could reach the public.

It is my contention that any subject, any idea, can be communicated effectively to the public.

What message do you want to get to the public about water quality management?

Step No. 1. Do not open with the phrase water quality management.

This outline was prepared for the Training Program by Marsha M. Myers, former Publication Editor of the Training Program, SEC.

WATER QUALITY SURVEYS

I INTRODUCTION

A Theoretical Approach

1 This discussion will present as a base, the procedures for the conduct of an ideal water quality survey.

B Practical Limitations

1 In practice, limitations of personnel, facilities, time and money, always will require compromise between the ideal and the possible.

C Objectives of Quality Studies

1 The four basic objectives behind the almost limitless number of possible reasons for water quality studies are:

- a Determination of the natural water quality of the stream.
- b Measurement, in a selected and limited period of time, of the existing effects of wastes on water quality and uses.
- c Procurement of data on waste loads, water quality and stream characteristics that will permit projection of the data to describe probable water quality and effects on uses under a variety of conditions other than those that prevailed during the study.
- d Determination of corrective measures needed to protect the stream water quality for proper uses.

II PRELIMINARY PLANNING

A Available Information

Assemble and review all readily available maps, information, and reports bearing on the stream under consideration.

B Problem and Objectives of Study

1 Define the problem requiring study as completely as possible on the basis of available information.

2 Prepare a list covering all known objectives that may be achieved by the study.

C Tentative Plan of Study

1 Prepare a brief preliminary plan of study for guidance in the subsequent field reconnaissance.

2 Include in the preliminary plan:

- a Locations and strengths of known sources of waste
- b Locations of areas of water use and a list of legitimate water uses
- c Section of major stream and locations of important tributaries and unique features such as dams and points of major diversions
- d Possible sampling stations
- e Sampling frequency and number of samples at each station

f Types of laboratory determinations

g Existing stream gauging stations and additional points where stream flow data are needed.

h Other hydrological data needed and possible means of procurement

i Potential laboratory locations

j Special supplies and equipment

k Personnel requirements

l Approximate cost of field operations

III PRELIMINARY FIELD OPERATIONS

A Importance

This step is often neglected, but a small advance party of two or three experienced field men can obtain information and make preparations that will save time and money and avoid much confusion and possible error when the subsequent field study starts.

B Local Contacts

Contact local agencies and individuals who use or have knowledge of the stream, and assemble available maps, reports, operating records, and verbal information.

C Sources of Wastes

1 Sewage

a Obtain best available estimate of sewered population.

b Examine sewage treatment plant, if any, and obtain copies of operating records on volumes and characteristics of sewage.

c Locate all points of discharge and make any installations necessary for sampling and gauging.

2 Industrial wastes

a Investigate and prepare flow diagram of process with especial reference to points of water use and waste discharge, and quantities of raw materials and finished products.

b Obtain information on plant operating schedule, with especial attention to daily or seasonal variations, and to any anticipated changes in process or increase in production.

c Obtain data on water consumption, numbers of plant employees by work shifts, and disposal of domestic sewage.

d Investigate waste treatment facilities, if any, and obtain operating records on volumes and characteristics of wastes.

e Locate all points of waste discharge, trace origins in plant process, and make any installations necessary for sampling and gauging.

f Collect samples for preliminary examination in laboratory to permit selection of proper analytical procedures.

D Stream

1 Observe entire reach of stream involved by wading, walking bank, or boat.

2 Note especially points of waste discharge and dispersion patterns, visual evidences of pollution confluences and mixing of tributaries, stream flow characteristics, approximate widths and depths, and locations of prospective sampling stations, and of water uses.

3 Collect bottom samples of biological organisms and bottom deposits, and observe evidences of algae.

a Bottom organisms serve as an index of degree of pollution, and of length of river that should be sampled.

b Bottom deposits indicate extent of sludge deposits.

c Algae indicates probable significance of photosynthesis and possible need for night sampling or light-dark bottle technique for full evaluation of dissolved oxygen variations.

4 Determine times of water travel between pertinent points on stream.

a Knowledge of times of water travel not only will permit the most intelligent conduct of study, but also is essential to the complete analysis, interpretation, and projection of data.

b Time and effort involved are a minor portion of the totals devoted to the stream study, and once established, need not be repeated in subsequent studies.

c Observations should be made at three or more stream flows to develop a time of travel versus stream discharge curve, which will permit interpolation for any desired stream flow.

d Methods include: tracing an inherent or added variable stream constituent; timing surface or submerged floats; and determining stream cross sections at selected intervals.

5 Select and identify sampling stations

a For DO evaluation on the majority of streams, there should be: one station just above each major waste source; stations at about half day intervals for two days time of travel below waste sources; and stations at about one day intervals for an additional three to four days time of travel.

b For coliform evaluation, the stations should be similar to those for DO except that the downstream stations at one day intervals should extend to a total of 10 to 20 days time of water travel below the source of wastes.

c In very small or very turbulent streams, natural purification may be well advanced in as little as one to two days time of water travel and stations should be at much closer intervals than suggested above.

d If tributary streams are important, there should be one station near the mouth of each tributary and one on the main stream just above each confluence.

e Stations should be established as close as practicable to points or areas of important water uses.

f At stations where wastes and tributary waters are well mixed, one sampling point near mid-channel usually is adequate.

g At stations where mixing is inadequate, common practice is to sample at the quarter points of the stream.

h Identify stations by adequate description and marking, and make any necessary preparations for use by sampler.

i Select best access routes to sampling stations and make a round-trip to stations to determine time required for sampling.

E Preliminary Stream Samples

1 Collect one or more sets of stream samples for preliminary examination:

a Dissolved oxygen results will be useful in establishing sampling stations and length of stream that should be studied b Determinations of coliform organisms and BOD will assist in selection of proper dilutions for subsequent use when study is started.

c Determinations of other constituents will enable analyst to select most acceptable laboratory procedures and to determine sizes of sample portions necessary for those analyses in which concentrations of constituents govern choice of portion sizes.

F Laboratory Location

Examine potential laboratory locations in relation to convenience to the field operations, adequacy of space and utilities, storage space, and revisions or additional facilities that will be needed.

G Miscellaneous

1 Locate local sources of supplies that will be purchased during study.

2 Determine transportation routes and schedules for shipments of samples, supplies, and materials.

3 Arrange for local personnel and special facilities that will be used.

4 Investigate rooming facilities convenient to the field operations.

IV FINAL PLANNING

A Revision of Preliminary Plan

With the preliminary plan as a guide, and with the knowledge gained from the preliminary field operations it will be possible to prepare an intelligent, workable plan for the field study.

B Objectives of Study

Redefine, add to, or delete from, the initial list of objectives, and prepare a set of specific objectives which will include provisions for all essential answers to the problem at hand, but will also eliminate needless expenditure, on nonessential matters, of effort which might better be devoted to the principal problem.

C Period of Field Operations

1 The overall period selected for the study should be at the time of year when past experience indicates that streamflows usually are relatively stable.

2 For evaluation of DO depletion; a period of warm weather and low streamflow is desirable.

3 For evaluation of coliform contamination, a period of intermediate flow may be desirable.

4 A period of intensive round-the-clock sampling for a few days generally is preferable to a period of a month of sampling daily or on alternate days, but a combination of both methods may be better than either one.

D Sampling

1 General

a If sources of wastes do not vary significantly during period of study, the wastes may be sampled in advance of the stream study.

b Sampling procedures and preservation of samples should follow "Standard Methods for the Examination of Water and Wastewater."

2 Sewage

a Collect samples around-the-clock at 15 minute to 1 hour intervals for three to seven days.

b Composite sample portions in portion to flow for three to four equal time periods of each day.

3 Industrial wastes

a In general, the better known the process is, and the less variable the waste discharge, the less detailed need the sampling be.

b For well known processes with little variation, equal sample portions collected at one-half to one hour intervals and composited for eight hours or for a complete cycle of operation on three to five days should be adequate.

c For little known and variable processes, detailed and prolonged sampling may be necessary, with samples collected at 5 to 15 minute intervals, and composited in proportion to flow for six to 24 hour periods for seven or more days or complete cycles of operation.

4 Stream

a If streamflow, waste discharge, or oxygen production by algae vary widely throughout the day, sample around-the-clock at several stations for at least one or two days to establish the daily cycle of variation in waste constituents and effects.

b In most streams, grab samples each day or on alternate days for two weeks to a month is adequate if conditions remain stable.

c The total number of samples collected from a single station during a field study generally ranges between 15 and 20. In the absence of wide variations in streamflow, 12 to 15 sets of samples usually yield adequate, usable results.

d If sampling is not around the clock, sample collection should be varied throughout the day as much as is feasible, and as a minimum, the direction of the sampling trip should be reversed on alternate days.

e When more than one point is sampled at a station, individual field determinations are made and a separate bacteriological sample is taken at each point, but samples for other determinations may be combined in a single composite. Bacteriological samples from two or more points at the same station may be composited in the laboratory.

E Gauging

1 Methods of waste and stream gauging are covered in another reference outline.

2 Generally stream discharge records are obtained from existing U. S. Geological Survey stations, or from special stations established and rated by them upon request.

3 Streamflow data at each main stream sampling station generally can be computed with sufficient accuracy from records for one or two main stream gauging stations and one for each major tributary.

F Laboratory Operations

1 Select principal determinations that will measure pertinent waste constituents and their effects, and auxilliary determinations that will contribute to interpretation of principal results.

2 Reject determinations that would be "interesting" but would not contribute to solution of the problem.

3 Generally useful principal determinations common to many stream studies are those for coliform bacteria, bottom organisms, temperature, DO, and BOD, and auxiliary determinations frequently are for pH, alkalinity, and turbidity.

Other determinations must be selected for special purposes and for special types of wastes.

5 Laboratory methods should follow procedures recommended in "Standard Methods for the Examination of Water, and Wastewater."

6 Avoid excessive overload of the laboratory by working closely with the

laboratory supervisor during the planning stage and accepting his estimate of the volume of work that can be handled.

7 In establishing the laboratory work load, allow time for calculation of analytical results at the end of each day.

8 Ship samples to the headquarters laboratory for all determinations of stable or preserved constituents that do not have to be made in the field.

G Personnel

1 The field crew should possess competencies for the specific tasks involved, or should be trained in advance if necessary.

2 Personnel needed commonly will include:

a Sanitary engineers

b Chemists

c Bacteriologists

d Biologists

e Sampling and laboratory aides

H Supplies and Equipment

1 Prepare and use a check list of needed field supplies and equipment.

2 All operations that can be performed in advance at headquarters, such as training, purchases, equipment repair, and reagent preparation will save money and valuable time in the field.

I Cost Estimate

1 Revise the initial estimate of cost of field operations in light of the final plan adopted.

2 Adjust the final plan to fit the money available for the study, or arrange for the additional money needed.

V FIELD OPERATIONS

A Briefing

1 Assemble the field crew and explain the problem, objectives, and details of field operations.

2 Take at least key personnel on a tour of pertinent physical features of the study.

3 Specify responsibilities of individuals for the various phases of the operation.

B Communications

Arrange a system of communication by which any member of the crew can be contacted within a reasonable length of time.

C Records

1 A few simple field and laboratory forms will serve to systematize the maintenance of basic records.

2 Keep all permanent field and laboratory records in bound volumes.

3 Encourage the recording of other than routine observations by both sampling and laboratory personnel.

4 Write down at once, and do not depend on memory for all significant observations.

D Revision of Operations

1 Review all daily data accumulated at the end of each day, and note especially any omissions or unusual results.

2 On the basis of the daily review, consider the need to:

   a Add new determinations if needed.

   b Omit determinations that are not showing significant results.

   c Revise analytical methods.

   d Change sampling stations or scheduled times of sampling.

   e Investigate causes of apparently abnormal or erroneous data.

3 Do not revise operations unless the data or other new information indicates changes are essential to achieve the desired objectives.

VI USE OF DATA

A Analysis and Interpretation

1 The raw data are of only limited value until submitted to analysis and interpretation by an experienced person who is familiar with the problem involved.

2 Methods of analysis and interpretation constitute a major subject in themselves and are covered by other lectures.

3 No amount of statistical manipulation of the data can produce sound conclusions unless the stream study is soundly conceived, and carefully and conscientiously executed.

B Report

A report should be prepared if the data are to achieve their maximum usefulness, since raw data in dead files only rarely benefit anyone.

C Ultimate Disposal

After use, the data should be filed, indexed, and described so that they can be available and understandable years later to others not directly associated with the study.

D Maximum Usefulness

The results of a stream pollution study yield their maximum benefit and return their greatest satisfaction to those who worked to obtain them only when the results serve as a basis for actual correction of an abuse of stream water quality.

REFERENCE

Kittrell, F.W. A Practical Guide to Water Quality Studies of Streams. USDI. FWPCA. CWR-5. 1969.

This outline was prepared by Francis W. Kittrell, former Special Consultant, National Field Investigations Center, --Cincinnati Center, OWP, EPA, Cincinnati, Ohio 45268

APPLICATION OF BIOLOGICAL DATA

I ECOLOGICAL DATA HAS TRADITIONALLY BEEN DIVIDED INTO TWO GENERAL CLASSES:

A Qualitative - dealing with the taxonomic composition of communities

B Quantitative - dealing with the population density or rates of processes occurring in the communities

Each kind of data has been useful in its own way.

II QUALITATIVE DATA

A Certain species have been identified as:

1 Clean water (sensitive) or oligotrophic

2 Facultative, or tolerant

3 Preferring polluted regions (see: Fjerdinstad 1964, 1965; Gaufin & Tarzwell 1956; Palmer 1963, 1969; Rawson 1956; Teiling 1955)

B Using our knowledge about ecological requirements the biologist may compare the species present

1 At different stations in the same river (Gaufin 1958) or lake (Holland 1968)

2 In different rivers or lakes (Robertson and Powers 1967)

or changes in the species in a river or/lake over a period of several years. (Carr & Hiltunen 1965; Edmondson & Anderson 1956; Fruh, Stewart, Lee & Rohlich 1966; Hasler 1947).

C Until comparatively recent times taxonomic data were not subject to statistical treatment.

III QUANTITATIVE DATA: Typical Parameters of this type include:

A Counts - algae/ml; benthos/m^2; fish/net/day

B Volume - mm^3 algae/liter

C Weight - dry wgt; ash-free wgt.

D Chemical content - chlorophyll; carbohydrate; ATP; DNA; etc.

E Calories (or caloric equivalents)

F Processes - productivity; respiration

IV Historically, the chief use of statistics in treating biological data has been in the collection and analysis of samples for these parameters. Recently, many methods have been devised to convert taxonomic data into numerical form to permit:

A Better communication between the biologists and other scientific disciplines

B Statistical treatment of taxonomic data

C In the field of pollution biology these methods include:

1 Numerical ratings of organisms on the basis of their pollution tolerance

(saprobic valency: Zelinka & Sladecek 1964)

(pollution index: Palmer 1969)

2 Use of quotients or ratios of species in different taxonomic groups (Nygaard 1949)

3 Simple indices of community diversity:

a Organisms are placed in taxonomic groups which behave similarly under the same ecological conditions. The number of species in these groups found at "healthy" stations is compared to that found at "experimental" stations. (Patrick 1950)

b A truncated log normal curve is plotted on the basis of the number of individuals per diatom species. (Patrick, Hohn, & Wallace 1954)

c Sequential comparison index. (Cairns, Albough, Busey & Chanay 1968). In this technique, similar organisms encountered sequentially are grouped into "runs".

$$SCI = \frac{\text{runs}}{\text{total organisms examined}}$$

d Ratio of carotenoids to chlorophyll in phytoplankton populations:

OD_{430}/OD_{665} (Margalef 1968)

OD_{435}/OD_{670} (Tanaka, et al 1961)

e The number of diatom species present at a station is considered indicative of water quality or pollution level. (Williams 1964)

f $\dfrac{\text{number of species (S)}}{\text{number of individuals (N)}}$

g $\dfrac{\text{number of species (S)}}{\text{square root of number of individuals } (\sqrt{N})}$

h $\dfrac{S - 1}{\log_e N}$ (Menhinick 1964)

i $d = \dfrac{\Sigma n_i (n_i - 1)}{N (N - 1)}$ (Simpson 1949)

where n_i = number of individuals belonging to the i-th species, and

N = total number of individuals

j Information theory:

The basic equation used for information theory applications was developed by Margalef (1957).

$$I = \frac{1}{N} \log_2 \frac{N!}{N_a!\, N_b! \ldots N_s!}$$

where I - information/individual; N_a, N_b...N_s are the number of individuals in species a, b, ... s, and N is their sum.

This equation has also been used with:

1) The fatty acid content of algae (McIntire, Tinsley, and Lowry 1969)

2) Algal productivity (Dickman 1968)

3) Benthic biomass (Wilhm 1968)

REFERENCES

1 Cairns, J., Jr., Albough, D.W., Busey, F, and Chaney, M.D. The sequential comparison index - a simplified method for non-biologists to estimate relative differences in biological diversity in stream pollution studies. J. Water Poll. Contr. Fed. 40(9):1607-1613. 1968.

2 Carr, J.F. and Hiltunen, J.K. Changes in the bottom fauna of Western Lake Erie from 1930 to 1961. Limnol. Oceanogr. 10(4):551-569. 1965.

3 Dickman, M. Some indices of diversity. Ecology 49(6):1191-1193. 1968.

4 Edmondson, W.T. and Anderson, G.C. Artificial Eutrophication of Lake Washington. Limnol. Oceanogr. 1(1):47-53. 1956.

5 Fjerdingstad, E. Pollution of Streams estimated by benthal phytomicro-organisms. I. A saprobic system based on communities of organisms and ecological factors. Internat'l Rev. Ges. Hydrobiol. 49(1):63-131.1964.

6 Fjerdingstad, E. Taxonomy and saprobic valency of benthic phytomicro-organisms. Hydrobiol. 50 (4):475-604. 1965.

7 Fruh, E.G., Stewart, K.M., Lee, G.F. and Rohlich, G.A. Measurements of eutrophication and trends. J. Water Poll. Contr. Fed. 38(8):1237-1258. 1966.

8 Gaufin, A.R. Effects of Pollution on a midwestern stream. Ohio J. Sci. 58(4):197-208. 1958.

9 Gaufin, A.R. and Tarzwell, C.M. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. Sew. Ind. Wastes. 28(7):906-924. 1956.

10 Hasler, A.D. Eutrophication of lakes by domestic drainage. Ecology 28(4):383-395. 1947.

11 Holland, R.E. Correlation of Melosira species with trophic conditions in Lake Michigan. Limnol. Oceanogr. 13(3):555-557. 1968.

12 Margalef, R. Information theory in ecology. Gen. Syst. 3:36-71. 1957.

13 Margalef, R. Perspectives in ecological theory. Univ. Chicago Press. 1968.

14 McIntire, C.D., Tinsley, I.J. and Lowry, R.R. Fatty acids in lotic periphyton: another measure of community structure. J. Phycol. 5:26-32. 1969.

15 Menhinick, E.F. A comparison of some species - individuals diversity indices applied to samples of field insects. Ecology 45:859. 1964.

16 Nygaard, G. Hydrobiological studies in some ponds and lakes. II. The quotient hypothesis and some new or little-known phytoplankton organisms. Klg. Danske Vidensk. Selsk. Biol. Skrifter 7:1-293. 1949.

17 Patten, B.C. Species diversity in net plankton of Raritan Bay. J. Mar. Res. 20:57-75. 1962.

18 Palmer, C.M. The effect of pollution on river algae. Ann. New York Acad. Sci. 108:389-395. 1963.

19 Palmer, C.M. A composite rating of algae tolerating organic pollution. J. Phycol. 5(1):78-82. 1969.

20 Patrick, R., Hohn, M.H. and Wallace, J.H. A new method for determining the pattern of the diatom flora. Not. Natl. Acad. Sci., No. 259. Philadelphia. 1954.

21 Rawson, D.S. Algal indicators of trophic lake types. Limnol. Oceanogr. 1:18-25. 1956.

22 Robertson, S. and Powers, C.F. Comparison of the distribution of organic matter in the five Great Lakes. in: J.C. Ayers and D.C. Chandler, eds. Studies on the environment and eutrophication of Lake Michigan. Spec. Rpt. No. 30, Great Lakes Res. Div., Inst. Sci. & Techn., Univ. Michigan, Ann Arbor. 1967.

23 Simpson, E.H. Measurement of diversity. Nature (London) 163:688. 1949.

24 Tanaka, O.H., Irie, S. Izuka, and Koga, F. The fundamental investigation on the biological productivity in the Northwest of Kyushu. I. The investigation of plankton. Rec. Oceanogr. W. Japan, Spec. Rpt. No. 5, 1-57. 1961.

25 Teiling, E. Some mesotrophic phytoplankton indicators. Proc. Intern. Assoc. Limnol. 12:212-215. 1955.

26 Wilhm, J. L. Comparison of some diversity indices applied to populations of benthic macroinvertebrates in a stream receiving organic wastes. J. Water Poll. Contr. Fed. 39(10):1673-1683. 1967.

27 Wilhm, J. L. Use of biomass units in Shannon's formula. Ecology 49:153-156. 1968.

28 Williams, L. G. Possible relationships between diatom numbers and water quality. Ecology 45(4):810-823. 1964.

29 Zelinka, M. and Sladecek, V. Hydrobiology for water management. State Publ. House for Technical Literature, Prague. 122 p. 1964.

This outline was prepared by C. I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

SECTION E

LEGAL AND JURISDICTIONAL ASPECTS OF WATER QUALITY

	Outline Number
Case Preparation and Courtroom Procedure	E-1
Water Quality Criteria	E-2
Research Needs: Fish, Other Aquatic Life and Wildlife	E-3

CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

- 1 Rule making
  - a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates
  - b Factors of safety and absolute prohibitions may be appropriate
- 2 Adjudications
  - a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

- 1 Civil in behalf of state or federal government
  - a Actions to compel action or suspension of action - nuisance, health hazard, etc., --including court action following federal conference --hearing procedure
  - b Violations of Water Quality Standards
  - c Violations of Effluent Standards or discharge permits
  - d Tort or contract actions relating to design and/or operation of treatment facilities
- 2 Criminal (dependent on content of applicable statutes)
  - a Discharge of specific materials
  - b Discharges from specific industries
  - c Littering
  - d Discharges harmful to fish and/or crustaceans
  - e Discharges harmful to specific types of receiving waters
  - f Discharges of poisons

    NOTE--In some of these situations doing the act may constitute the violation; in others proof of intent or knowledge of effects may also have to be proved.
- 3 Private actions for damages or to compel action.
  - a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

- 1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

- 1 Facts - direct
  - a The material was floating from the outfall.
- 2 Derived values - expert testimony - test results and/or opinion as to effects
  - a The D.O. was zero; the waterway was polluted; the plant can be built in 6 months.
- 3 Hearsay
  - a Joe told me

4 Relevancy

5 Admissibility vs. weight

a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

1 Sampling

a Chain of custody

b Tags, etc.

c Containers

d Place and time

e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering; and availability of portions for analysis by other side (non-transitory criteria)).

2 Analysis

a Who performed (Can identity of each participant be shown?)

b Admission through supervisor - custodian

c Scientific acceptance of method. Is there a particular method required to be used by the agency?

d Propriety of conduct

e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).

3 Tests

a Comparison with actual conditions

b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

1 Who has special knowledge - and of what particular areas?

2 Indicators

3 Significance of numerical determinations or observations

4 Consistency with own prior publications and testimony

5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.

6 Use of treatises

G Conduct on the Witness Stand

1 General

a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.

b Use layman's language to extent possible.

c Listen to question and answer it to best of your ability.

d Speak so that court reporter, judge, jury, and counsel can hear you.

e Speak in language that will be understood; don't talk down.

f Answer only what you are asked --don't volunteer; however, answer with precision.

g There is nothing wrong with asking to have a question repeated or rephrased.

h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

- i There is nothing wrong with thinking out your answer before responding.
- j You are not expected to know all the answers--if you do not know, admit it.
- k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidimeological inferences from results).
- l Don't try to answer before the judge rules on objection.
- m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.
- n Don't be afraid to admit what may appear to be damaging.

2 If you are testifying as an expert:

- a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.
- b Differentiate between physical facts (measurements and observations) and opinion (derived values).
- c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences.
- d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.
- e If you're being paid to testify, admit it.

3 Scientific personnel as advisers to counsel:

- a Review and refamiliarize self with materials before you discuss with your attorney.
- b Be in a position to present all facts known to you simply and concisely: Who, What, When, Where, and Why, How.
- c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.
- d Use of standard report forms
- e Ability to recommend additional witnesses with needed specialized knowledge
- f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results
- g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.

H Non-Verbal Presentation of Evidence

- 1 Exhibits - including photographs
- 2 Summaries
- 3 Business and/or government records
  - a Prepared contemporaneously and in usual course of activities
- 4 Pre-prepared direct examination
  - a Usually limited to actions before ICC, FPC, and other federal agencies.

I Criminal Procedure

- 1 Privilege Against Self Incrimination (available only to persons)
  - a Warning and suspects
  - b Effect of duty to report spills

c Effect of duty to obtain license or permit and/or furnish operating reports

d Immunity from prosecution

2 Double Jeopardy

3 Unreasonable search and seizure

a Available to persons and corporations

4 Procedures and need for arrest and search warrants -- possible cause

---

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Administrative & Court Proceedings, and Excerpts from Revised Draft of Proposed Rules of Evidence for the United States Courts can be found on the following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

Court or Agency	Fact Finder	Burden of Proof	Comments
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence.		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed
RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.
PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.
PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.
EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.
MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE; IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any; his deposition may be taken by any party; and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

(a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.

(b) Declarant. A "declarant" is a person who makes a statement.

(c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness:

(5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.

(6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.

(18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision. The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule:

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated.

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT; ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eyewitnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place. The judge may order that they be produced in court.

WATER QUALITY CRITERIA

The chemical analysis of water, in a large part, determines the beneficial uses of a given body of water. These uses include water supplies for drinking, industry, irrigation, stock and wildlife, and fish and aquatic life.

The chemicals present and physical state of the water which affect its uses include: suspended solids, dissolved solids, temperature, hardness, phenol, cyanides, nitrates, sulfates, zinc, copper, iron, lead, nickel, manganese, magnesium, selenium, and boron.

I SUSPENDED SOLIDS

A Source

In natural waters, suspended solids normally consist of erosion silt, organic detritus and plankton.

B Effect on Beneficial Uses

1 Domestic water supplies

The 1962 PHS standard for drinking water did not specify limiting concentration for suspended solids. It is controlled by the limits on turbidity (5 units) and total solids (500 mg/l). It has been proposed that for drinking water of ideal quality, suspended matter should be limited to 0.1 mg/l, or less.

2 Industrial water supplies

Suspended solids cause foaming in boilers; incrustation on equipment exposed to water. They are undesirable in water used by textile industries, paper and pulp mills, beverages or dairy product plants, cooling systems and power plants, or in water for steel manufacturing.

3 Irrigation water

Paperboard mill wastes containing 240 mg/l of suspended solids have been successfully used for spray irrigation of alfalfa.

4 Fish and aquatic life

a Suspended solids may kill fish, shellfish, and various aquatic fauna, by clogging the gills and respiratory passages.

b Suspended solids blanket the stream bottom, killing eggs, young and food organisms. They destroy spawning beds.

c They screen out light (turbidity). It is recommended that all cellulose pulps and sawdust be excluded from streams.

d It is also recommended that mineral suspended solids be so finely divided as to pass through a 1000-mesh screen, and that the stream bottom not be blanketed to a depth of more than one-quarter inch.

II DISSOLVED SOLIDS

A Source

Dissolved solids consist mainly of carbonates, bicarbonates, chlorides, sulfates, phosphates, and possibly nitrates of calcium, magnesium, sodium, or potassium, with traces of manganese and iron.

B Effects on Beneficial Uses

1 Domestic water supplies

PHS drinking water standards specify the total dissolved solids should not exceed 500 mg/l. This limit was set primarily on the basis of taste thresholds. Higher concentrations may be consumed without harmful physiological effects.

2 Industrial water supplies

Dissolved solids cause foaming in boilers and interfere with clearness, color, and taste of many finished products.

3 Irrigation water

It has been sonsidered that 1000 mg/l is a concentration which approaches the limit for best crop growth. Irrigation waters in California have been classified according to salt content, as follows:

Classification	Concentration mg/l
Excellent	175, or less
Good	175 - 525
Permissible	525 - 1400
Doubtful	1400 - 2100
Injurious	2100, or more

4 Stock and wildlife

A maximum permissible concentration of dissolved solids in water of 2500 mg/l has been recommended.

In Montana, livestock standards are:

Good	- 2500 - 3500 mg/l
Poor	- 3500 - 4500 mg/l
Unsatisfactory	- 4500 mg/l, over

5 Fish and aquatic life

The blood of freshwater fishes has an osmotic pressure approximately equal to six atmospheres, or about 7000 mg/l as sodium chloride.

In practical pollution work, any effluent with an osmotic pressure greater than six atmospheres may be expected to be deleterious to freshwater fish.

Fish in waters of low salinity can not survive sudden exposure to high salinities, such as those resulting from discharge of oil well brines.

Dissolved solids may influence the toxicity of heavy metals and organic compounds to fish and other aquatic life because of the antagonistic effect of hardness metals.

6 Summary

Use	Dissolved Solid
Domestic Water Supply	1000 mg/l
Irrigation	700 mg/l
Stock watering	2500 mg/l
Freshwater fish	2000 mg/l

III HARDNESS

A Source

Hardness in water may be caused by the natural accumulation of salts of Ca and Mg, from contact with soil and geological formations, or it may enter from direct pollution by industrial and commercial operations.

B Effects on Beneficial Uses

1 Domestic water supplies

In good water, the total hardness is usually below 250 mg/l. Hardness above 500 mg/l is considered unsuitable for general domestic purposes. The major detrimental effect of hardness is economics.

The annual cost of cleansing agents for a family of five, doubles as the hardness increases from zero to 375 mg/l. It has been found that the savings for soap, scouring compounds, synd ets and bleaches, were $1.15 per capita per year for each 100 mg/l reduction in hardness.

2 Industrial water supplies

Excessive hardness is undesirable in water for laundries, carbonated beverages, metal finishing, dyeing and textiles, food, paper, pulp, etc.

3 Irrigation water

Hard water is superior to soft water for irrigating.

4 Fish and aquatic life

In hard water, toxic metals may be less dangerous.

IV TEMPERATURE

A Sources and Effects

Temperature changes in bodies of water may result from natural climatic phenomena or from the introduction of industrial wastes. Temperature is important and sometimes critical for many uses of water.

Increased temperature may cause decreased oxygen capacity, increased oxygen demand, putrefaction of sludge deposits, and growth of sewage fungus. Stream temperature may also be increased by irrigation. Increases from 10° to 20° C have been reported in the temperature of irrigation return water.

B Effects on Beneficial Uses

1 Domestic water supplies

a Water temperature of 10° C is usually satisfactory for drinking purposes. Temperatures of 15° C or higher are usually objectionable.

b The efficiency of water purification and treatment is better in warm rather than in cool water.

c Chlorination is more effective in summer than in winter. Bactericidal effects of disinfectants are generally increased by an increase in the temperature of the water.

d The alum dose required to reduce the color of raw water, however, was observed to be as much as two times greater at 14°- 24° C than at 8° - 14° C.

2 Industrial water supplies

a Water for barley malting should have a temperature of about 12° C.

b Water temperature for use in dairy processing should be about 16° C, or less.

c Water temperature for use in steel mills should be below 24° C.

d Streams have lost much value for cooling as the water temperature rises to as high as 27° C during the course of discharge and re-use of the water by many industries in succession.

3 Irrigation water

Below 10° C, water weeds grow very sparsely; between 10° - 15° C, growth is prolific.

4 Fish and aquatic life

a Warm temperatures reduce solubility of dissolved oxygen, increase metabolism, respiration and oxygen demand of fish.

b Toxicity of many substances is intensified as the temperature rises.

c There is a maximum temperature that each species of organism can tolerate.

d ORSANCO has recommended that waste discharges be so controlled that the temperature of the receiving water not be raised above 34° C at any one place, and not above 23° C at any place during the months of December through April; and not be raised at all in streams suitable for trout propagation.

V PHENOL

A Source

Phenolic wastes arise from the distillation of wood, from gas works, coke ovens, oil refineries, chemical plants, sheep dips, and from human and animal refuse.

B Effects on Beneficial Uses

1 Domestic water supplies

PHS drinking water standards limit the concentration of phenolic compounds to 0.001 mg/l because of the taste resulting from the action of chlorine on phenolics in water.

2 Industrial water supplies

Phenol is deleterious in many of the food and beverage industries and may cause obnoxious tastes and odors.

3 Irrigation water

Phenol in irrigation water is not considered to be deleterious to crops.

4 Stock and wildlife

Appreciable concentrations of phenol are not toxic to animals.

5 Fish and aquatic life

a Information relating to pure phenol indicates that 24, 48, and 96 hour TL_m concentrations are in the general range of 10 - 20 mg/l at 20° C. Phenol appears to act as a nerve poison, causing too much blood to get to the gills and to the heart cavity of the fish.

b Threshold responses to phenol occur in the vicinity of 1.0 mg/l.

c Chlorophenols produce a bad taste in fish flesh even at concentrations far below lethal or toxic doses.

d Summary

Supply	Phenol Concentration
Domestic	0.001 mg/l
Irrigation	50.0 mg/l
Livestock	1000.0 mg/l
Fish and aquatic life	0.20 mg/l

VI CYANIDES

A Source

Cyanides occur in effluents from gas works and coke ovens, from the scrubbing of gases at steel plants, from metal cleaning and electroplating processes and from chemical industries. HCN is largely undissociated at pH values of 8 and less.

B Effects on Beneficial Uses

1 PHS drinking water standards set a recommended limit of 0.01 mg/l and a mandatory limit of 0.02 mg/l. The odor threshold for HCN in water is approximately 0.001 mg/l. The CN^- ion is changed rapidly to the relatively non-toxic sulfur complex (SCN^-) thiocyanate in the liver.

2 Industrial water supplies

No information regarding industrial water.

3 Irrigation water

No information regarding effects of cyanide on irrigation water.

4 Fish and aquatic life

a The toxicity of CN^- is affected by pH and temperature - a rise of 10° C produces a two- to three-fold increase in the rate of lethal action.

b Nickel may complex with cyanide to reduce lethality, but zinc and cadmium cyanide complexes are exceedingly toxic.

c Cyanide inhibits oxidase responsible for the transferring of oxygen from the blood.

VII NITRATE

A Source

In spite of the many sources, nitrates are seldom abundant in natural surface waters for they serve as an essential fertilizer for all types of plants from phytoplankton to trees. Photosynthetic action is constantly utilizing nitrates and converting them to organic nitrogen in plant cells.

In ground waters (absence of photosynthesis) excessive and deleterious concentrations of nitrates are often found.

B Effects on Beneficial Uses

1 Domestic water supplies

It has been widely recommended that water containing more than 10 mg/l of nitrate nitrogen should not be used for infants.

Nitrates are rated among the poisonous ingredients of mineralized waters, with potassium nitrate being more poisonous than sodium nitrate. Excess nitrates cause irritation of the mucous linings of the gastrointestinal tract and bladder.

2 Industrial water supplies

Nitrates and free nitric acid at concentrations of 15 - 30 mg/l tend to lower pH and cause corrosion.

3 Irrigation water

Nitrates are desirable for their fertilizing value.

4 Stock and wildlife

Special attention should be paid to the concentration of nitrates in stock waters, especially when total salt concentration exceeds 570 - 1000 mg/l

5 Fish and aquatic life

By increasing plankton growth and the development of fish food organisms, nitrates indirectly foster increased fish production.

6 They may delay septicity through the available oxygen in NO_3 ion.

VIII SULFATES

A Source

Sulfates occur naturally in waters, particularly in the western United States, as a result of leachings from gypsum and other common minerals. It occurs as the highest oxidized stage of sulfides, sulfites and thiosulfates.

Iron pyrite FeS, may be leached from abandoned coal mines, and the sulfide ions converted in surface streams to sulfates.

Sulfates may be discharged in numerous industrial wastes, such as those from tanneries, sulfate-pulp mills, or textile mills.

B Effects on Beneficial Uses

1 Domestic water supplies

The PHS 1962 drinking water standards recommend 250 mg/l.

2 Industrial water supplies

Sulfates increase the corrosiveness of water toward concrete.

3 Irrigation water

a Sulfates are somewhat less toxic than chlorides in irrigation waters.

b Sulfates can cause the precipitation of calcium and may be toxic to plants.

c Good irrigation water contains less than 190 mg $SO_4^{=}$/l. More than 950 mg/l makes it unsuitable for use.

4 Stock and wildlife

It has been observed that cattle taking drinking water containing 3590 mg/l and 2104 mg/l of sulfate, died. A threshold limit of 1000 mg/l has been suggested.

5 Fish and aquatic life

In the U.S., 95% of the waters supporting good game fish contain less than 90 mg sulfate/l.

6 Summary

It appears that the following concentration of sulfates will not be detrimental for the indicated beneficial use.

Domestic Water Supply	500 mg/l
Irrigation Water	200 mg/l
Stock Watering	500 mg/l

IX ZINC

A Source

Zinc occurs abundantly in rocks and ores. It is used extensively for galvanizing, in alloys, for electrical purposes, in printing plates, for dye manufacturing. Zinc salts are used in paint pigments, cosmetics, pharmaceuticals, dyes, insecticides.

In zinc mining areas, zinc has been found in natural waters in concentrations as high as 50 mg/l in effluents from metal plating works.

B Effects on Beneficial Uses

1 Domestic water supplies

a The taste threshold for zinc occurs at about 5 mg/l. Zinc has no adverse physiological effects upon man, except at very high concentrations.

b At a concentration of 30 mg/l, zinc has a milky appearance and causes a greasy film on boiling.

2 Industrial water supplies

Zinc-bearing water should not be used in acid drinks like lemonade, because zinc citrate and other organic zinc compounds which are formed can be poisonous.

3 Irrigation water

Small amounts of zinc are needed for nutrition by most crops, but toxicity results when concentration exceeds a very low level.

4 Fish and aquatic life

It is toward fish and aquatic organisms that zinc exhibits its greatest toxicity. In soft water, concentrations of zinc, ranging from 0.1 to 1.0 mg/l, have been reported to be lethal.

X COPPER

A Source

Copper salts occur in natural surface waters only in trace amounts up to about 0.05 mg/l, so that their presence is generally the result of pollution. Copper salts are used in textile processing, tanning, electroplating, insecticides and fungicides.

B Effects on Beneficial Uses

1 Domestic water supplies

The limiting factor in domestic water supplies is taste. Threshold concentrations for taste have been generally reported in the range of 1.0 - 2.0 mg/l of copper, while as much as 5.0 - 7.5 mg/l makes the water completely unpalatable.

2 Industrial water supplies

In food-preserving industries, copper causes undesirable color reactions, forming tannates and sulfides. Traces of copper in metal-plating baths affect the smoothness and brightness of the metal deposits.

3 Irrigation water

Minute quantities of copper are beneficial or essential for plant growth.

4 Fish and aquatic life

The sulfates of copper and zinc, copper and cadmium, are synergistic in their toxic effect on fish. Copper is concentrated by plankton by factors 1000 to 5000. Copper concentrations as low as 0.1 to 0.5 have been reported toxic to bacteria and other micro-organisms. Copper concentrations as low as 0.01 to 0.5 mg/l have interferred with BOD determinations.

5 Summary

Use	Recommended Copper limit
Domestic supply	1.00 mg/l
Irrigation water	0.10 mg/l
Fish and aquatic life	
Freshwater	0.02 mg/l
Seawater	0.05 mg/l

XI IRON

A Source

In addition to corrosion products, natural water may be polluted by iron-bearing industrial wastes, pickling operations, and mine drainage. Ferrous ions are readily oxidized in natural surface waters to the ferric condition, and form insoluble hydroxides. In well-aerated waters, the concentration of iron is seldom high. In ground waters the pH and Eh may be such that high concentrations of iron remain in solution.

B Effects on Beneficial Uses

1 Domestic water supplies

The taste threshold of iron in water has been given as 0.1 mg $FeSO_4$/l and 0.2 mg $Fe_2(SO_4)_3$/l.

2 Industrial water supplies

0.1 to 0.2 mg/l iron has been recommended as a limit.

3 Irrigation water

It is generally of little importance in irrigation practice.

XII LEAD

A Source

Low pH, high dissolved oxygen and high nitrate content favor lead solubility. Owing to the fact that the carbonate and hydroxide are insoluble and the sulfate is only sparingly soluble, lead is not likely to remain long in natural waters.

B Effects on Beneficial Uses

1 Domestic water supplies

Lead is a cumulative poison. It tends to be deposited in bone. The PHS mandatory limit for lead is 0.05 mg/l. The U. S. Government has also established a tolerance of lead in food at 7 mg/kg--more than 100 times the limit for drinking water.

2 Industrial water supplies

Traces of lead in metal-plating baths will affect the smoothness and brightness of deposits.

3 Irrigation water

Lead is apparently toxic to plants.

4 Stock and wildlife

It is not unusual for cattle to be poisoned by lead in water. Chronic lead poisoning among animals has been caused by 0.18 mg lead/l in soft water; 0.5 mg lead/l is the maximum safe limit for lead in water for animals.

5 Fish and aquatic life

In water containing lead salts, a film of coagulated mucus first forms over the gills and then over the whole body of the fish. The death of the fish is caused by suffocation due to this obstructive layer.

Calcium in concentration of 50 mg per liter destroys the toxic effect of 1.0 mg lead/l.

The dissolved content of surface waters should be restricted to 0.1 mg/l for drinking water and for fish and aquatic life.

XIII NICKEL

A Source and Behavior

Many nickel salts are highly soluble in water and since they are used in metal-plating works, metal finishing and mining, they may be discharged to surface or ground waters.

Metallic nickel does not merit serious consideration as a water pollutant, but nickel ions may be detrimental to use instead of beneficial.

B Effects on Beneficial Uses

1 Domestic water supplies

Toxicity of nickel to man is believed to be very low.

2 Irrigation water

Nickel is extremely toxic to citrus plants.

3 Stock and wildlife

Nickel appears to be less toxic to fish than copper or zinc.

Nickel combines readily with cyanide to form a nickel-cyanide complex which is relatively stable. In acid waters, however, this complex breaks down and releases HCN.

XIV MANGANESE

A Source

Like iron, it occurs in the divalent and trivalent forms. The chlorides, nitrates and sulfates are highly soluble in water; but the oxides, carbonates and hydroxides are only sparingly soluble.

For this reason, manganic (Mn^{+4}) or manganous (Mn^{+2}) ions are seldom present in natural surface waters in concentrations above 1.0 mg/l.

In ground water, subject to reducing conditions, manganese can be leached from the soil and occur in high concentrations.

B Effects on Beneficial Uses

1 Domestic water supplies

PHS drinking water standards set a recommended limit of manganese at 0.05 mg/l. This limit is based on aesthetic and economic considerations rather than physiological hazards.

It causes unpleasant tastes, leaves deposits on food during cooking, stains and discolors laundry and plumbing fixtures.

2 Industrial water supplies

Excessive manganese is undesirable in water for use in many industries including textiles, dyeing, food processing, etc. Primarily, manganese causes staining or poor color control.

3 Irrigation water

Manganese is essential for plant growth, apparently as an enzyme activator.

4 Stock and wildlife

Manganese is important in the nutrition of livestock, being closely related to that of calcium, phosphorus, iron and copper.

5 Fish and aquatic life

The permanganates are much more toxic to fish than the manganous salts.

Use	Concentration of Manganese
Domestic water supply	0.05 mg/l
Industrial water supply	0.05 mg/l
Irrigation water	0.50 mg/l
Stock and wildlife	10.00 mg/l
Fish and aquatic life	1.00 mg/l

XV MAGNESIUM

A Source

One of the most common elements in the crust of the earth is magnesium, about 2.1% of the earth's crust. With the exception of the hydroxide at high pH values, its salts are very soluble. Its carbonates will dissolve to the extent of 100 - 300 mg/l.

$$[Mg^{++}][OH^-]^2 = 1.2 \times 10^{-11} \text{ at } 18^\circ C$$

pH		Solubility of Magnesium ion, Mg^{++}
7	1200.0 moles/l	28,800 g/l *
10	1.2 moles/l	28.8 mg/l
11	0.012 moles/l	0.288 mg/l

* Calculated value

B Effects on Beneficial Uses

1 Domestic water supplies

At high concentrations, magnesium

salts have a laxative effect. Also, they increase hardness.

2 Industrial water supplies

Industry	Limit of Mg
Brewing	30 mg/l
Soda pulp	12 mg/l
Sugar making	10 mg/l
Textile manufacturing	5 mg/l

XVI SELENIUM

A Source

Selenium is used in a variety of industrial applications, such as pigmentation in paints, dyes and glass production, and in electrical apparatus. Selenium is highly toxic to man; symptoms are similar to those of arsenic poisoning.

B Effects on Beneficial Uses

1 Domestic water supplies

PHS drinking water standards have a mandatory limit of 0.01 mg/l.

2 Industrial water supplies

Little information is available.

3 Irrigation water

The classification of irrigation water based on selenium concentrations is as follows:

Irrigation Class	Selenium mg/l	Remarks
Low	0.00 - 0.10	No plant toxicity
Medium	0.11 - 0.20	Usable, but with long term accumulation
High	0.21 - 0.50	Toxic accumulation in plants
Very high	Over 0.50	Not usable under any condition

4 Stock and wildlife

Selenium poisoning causes "alkali disease or blind staggers". It occurs among cattle, sheep, pigs, and horses in both chronic and acute forms.

In water containing 0.4 - 0.5 selenium/l, it is believed to be non-toxic to cattle. Selenium content of feed is a more critical factor.

5 Fish and aquatic life

It is believed that selenium is passed up through the food chain to the fish which accumulates this element in the liver in lethal concentrations.

XVII BORON

A Source

Boron occurs as sodium borate (borax) or as a calcium borate in mineral deposits and natural waters of southern California and in Italy. Boric acid and boron salts are used extensively in industry for weatherproofing wood, fireproofing fabrics and manufacturing glass and porcelain.

B Effects on Beneficial Uses

1 Domestic water supplies

Boron in drinking water is not regarded generally as a hazard to human beings.

2 Irrigation water

Boron is an essential element in the nutrition of higher plants, yet concentrations in excess of 0.5 mg/l may be deleterious for certain crops.

3 **Stock and wildlife**

The lethal dose of boric acid for animals varies from 1.2 to 3.5 grams per kg of body weight.

4 **Fish and aquatic life**

Boric acid can be toxic.

REFERENCES

1 Standard Method for the Examination of Water and Wastewater. 12th Edition. 1965.

2 Water Quality Criteria. McKee and Wolf, Second Edition. The Resources Agency of California. State Water Quality Control Board, Sacramento, California.

This outline was prepared by J.W. Mandia, Chemist, Formerly with National Training Center, FWQA, Cincinnati, OH 45226.

fish, other aquatic life, and wildlife

(Extracted from: Report of the Committee on
WATER QUALITY CRITERIA
FWPCA, USDI, April 1, 1968)

letter from the chairman

THE MEMBERSHIP of the National Technical Advisory Subcommittee on Water Quality Criteria for Fish, Other Aquatic Life, and Wildlife represents training and experience in several phases of freshwater, marine and wildlife ecology, physiology, and toxicology. The task of this Subcommittee is to describe, insofar as possible, under present knowledge: (1) the environmental requirements of aquatic life and wildlife, (2) the environmental concentrations of potential toxicants that are not harmful under long-term exposure, and (3) to suggest indirect methods for determining safe concentrations through bioassays and application factors. Because present knowledge of environmental requirements is incomplete and information on safe concentrations of toxicants is nonexistent for most organisms, the recommendations for water quality criteria of necessity are incomplete, tentative, and subject to change as additional information becomes available. In the determination of these criteria, the Subcommittee has utilized the broad knowledge, the many years of experience, and the understanding and commonsense of the Subcommittee members.

In order to expedite this task, the Subcommittee was divided into three groups: one for freshwater organisms; one for marine and estuarine organisms; and a third for wildlife.

Six task forces were set up in each of the first two groups. Each of these task forces was assigned certain environmental factors to review present knowledge and determine environmental conditions essential for the survival, growth, reproduction, general well-being, and production of a desired crop of aquatic organisms. Members assigned to each task force were experts on that particular subject or had wide experience with the factors or materials in question. They were selected with this in mind so that the whole subject could be covered most effectively. The composite report thus prepared was reviewed by the full Subcommittee and approved on October 31 and November 1, 1967, in Washington, D.C.

CLARENCE M. TARZWELL,
Chairman.

introduction

DURING the course of geologic time, organisms which were able to adapt so they were better fitted to live under existing environmental conditions were the ones which survived and now form the biota. Geologic change is a slow process and biota developed which were adapted not only to the physical and chemical but also to the biological factors of the environment. The environmental factors to which organisms adapted through the evolutionary process are now their environmental requirements. Therefore, any relatively rapid change in these conditions can be detrimental or even disastrous. Because the biota is the result of long evolutionary processes during which delicate balances were established, a change in conditions or in a portion of the biota can have far reaching effects.

Man has now attained the ability to alter drastically his environment and that of other organisms. Many of his activities already have impaired seriously his own environment and that of other living things. Water pollution engineering works and other changes that modify the aquatic environment rank high in causing detrimental effects.

Water pollutants may be harmful through alterations in natural environmental conditions (such as temperature, dissolved oxygen, pH, carbonates, etc.), through physiological and other changes due to the addition of toxicants, or through both. Thus, in determining the effects of pollutants we must consider environmental, physiological, and accumulative effects.

Substances in suspension and solution, whether solid, liquid, or gas, largely determine the quality of the water. Aquatic organisms are affected not only directly by these materials, but also indirectly through their effects on other forms of aquatic life which comprise their food, competitors, and predators. Hence, the determination of water quality requirements for aquatic life is a very involved task. The problem is further complicated by the fact that different species and different developmental or life stages of the same or different species may differ widely in their sensitivity or tolerance to different materials, to ranges in environmental conditions, and to the cumulative synergistic and antagonistic effects of toxicants.

In determining water quality requirements for aquatic life and wildlife, it is essential to recognize that there are not only acute and chronic toxic levels but also tolerable, favorable, and essential levels of dissolved materials. Lethal, tolerable, and favorable levels and conditions may be ascertained by: (1) determining the environmental factors and concentrations of materials which are favorable in natural waters; (2) determining by laboratory studies the relative sensitivity of organisms to various environmental factors, and ranges which are tolerable and favorable; (3) determining by means of different bioassay studies the behavioral, physiological, and other responses of organisms to potential toxicants and concentrations of these materials which are not harmful under continuous exposure; and (4) testing laboratory findings in the field to determine their adequacy for the protection of aquatic and wildlife resources.

In approaching this problem of protecting our aquatic and wildlife resources, it must also be realized that: (1) certain natural complexes of dissolved materials to which aquatic organisms have become adapted are favorable whereas other concentrations or compositions may not be; (2) unnatural materials added by man can be unfavorable; (3) altering the amounts of substances normally found in the environment can be harmful; (4) toxicity is a quantitative term—any material becomes toxic when its concentration exceeds certain levels. It is essential, also, to realize that requirements must be maintained throughout periods of low water, maximum discharge, maximum temperature, minimum DO, variations in pH, turbidity, salinity, etc. Further, it should be understood that: (1) unfavorable conditions which may be resisted for long periods by adults may be entirely unfavorable for the survival of the species; (2) conditions need to be unfavorable for only a few hours to eliminate a population or group of species; and (3) levels of environmental factors and concentrations of toxicants that appear to cause no harm during a few hours of exposure may be intolerable for extended periods or for recurring short-term exposures.

In defining water quality requirements for aquatic life and wildlife, it is necessary to define the extreme upper and lower limits of the various environmental factors as well as the optimum values. These extremes are outer limits and constitute the minimum objectives to be obtained in the improvement of waters for aquatic life. It is not the intention of the Subcommittee that such levels are to be considered as satisfactory. Further, it is stressed that waters of higher quality should not be degraded towards approximation of the extremes. For example, the dissolved oxygen content of water should be near saturation for best production. The lower limits for oxygen indicated in the report, therefore, represents the objective to be obtained in the improvement of water, and not the level to which good waters may be lowered.

It is essential that the various recommendations be considered in context with the body of the report, taking due consideration of the variability of local conditions and native biota.

Within the United States there are great variations in environmental conditions and in the flora and fauna. The environmental requirements of the biota are different not only for different regions but for different portions of the same region. Overlying these differences are seasonal changes and daily variations that have become essential factors in the environment. Ideally, therefore, water quality criteria for aquatic life and wildlife should take into consideration local variations in requirements, seasonal changes, and daily variations. They should be national in scope. They should be applicable to streams of various size and character, to all types of lakes, to reservoirs, estuaries, and coastal waters.

It is obvious that more research is needed on the character, conditions, and interrelations in fresh water, marine, and estuarine ecosystems which are subjected to degradation or alteration as well as on the physiological requirements and tolerances of the various species involved in these different ecosystems. This need must be satisfied for the establishing of sound criteria to maintain and preserve aquatic resources and to permit the most economical and productive use of these resources by man.

Further, water quality requirements must be expressed so as to allow for environmental modifications where such modifications are justifiable and deemed to be in the public interest.

All these factors have been considered in developing the following recommended water quality requirements for aquatic life.

It is the purpose of this document to define the water quality requirements which must be met to insure a favorable environment for fish, other aquatic life, and wildlife. This report will do this by identifying those aspects of water quality that are most important in the light of current knowledge and quantifying them where possible. Where quantification is not yet possible, narrative guidelines will be offered. There is no doubt that the water quality requirements contained herein must be reviewed periodically and updated in the light of additional and improved scientific data. The recommendations given in this report are considered to be satisfactory for aquatic life. In all instances where natural conditions fall outside the recommended ranges, this environment may be marginal and should not be changed in such a way as to make it more unfavorable.

zones of passage

ANY BARRIER to migration and the free movement of the aquatic biota can be harmful in a number of ways. Such barriers block the spawning migration of anadromous and catadromus species. Many resident species make local migrations for spawning and other purposes and any barrier can be detrimental to their continued existence. The natural tidal movement in estuaries and downstream movement of planktonic organisms and of aquatic invertebrates in flowing fresh waters are important factors in the re-population of areas and the general economy of the water. Any chemical or thermal barrier destroys this valuable source of food and creates unfavorable conditions below or above it.

It is essential that adequate passageways be provided at all times for the movement or drift of the biota. Water quality criteria favorable to the aquatic community must be maintained at all times in these passageways. It is recognized, however, that certain areas of mixing are unavoidable. These create harmfully polluted areas and for this reason it is essential that they be limited in width and length and be provided only for mixing. The passage zone must provide favorable conditions and must be in a continuous stretch bordered by the same bank for a considerable distance to allow safe and adequate passage up and down the stream, reservoir, lake, or estuary for free-floating and drift organisms.

The width of the zone and the volume of flow in it will depend on the character and size of the stream or estuary. Area, depth, and volume of flow must be sufficient to provide a usable and desirable passageway for fish and other aquatic organisms. Further, the cross-sectional area and volume of flow in the passageway will largely determine the percentage of survival of drift organisms. Therefore, the passageway should contain preferably 75 percent of the cross-sectional area and/or volume of flow of the stream or estuary. It is evident that where there are several mixing areas close together they should all be on the same side so the passageway is continuous. Concentrations of waste materials in passageways should meet the requirements for the water.

The shape and size of mixing areas will vary with the location, size, character, and use of the receiving water and should be established by proper administrative authority. From the standpoint of the welfare of the aquatic life resource, however, such areas should be as small as possible and be provided for mixing only. Mixing should be accomplished as quickly as possible through the use of devices which insure that the waste is mixed with the allocated dilution water in the smallest possible area. At the border of this area, the water quality must meet the water quality requirements for that area. If, upon complete mixing with the available dilution water these requirements are not met, the waste must be pretreated so they will be met. For the protection of aquatic life resources, mixing areas must not be used for, or considered as, a substitute for waste treatment, or as an extension of, or substitute for, a waste treatment facility.

summary and key criteria

RECOMMENDATIONS given below are considered to be satisfactory for aquatic life. In all instances where natural conditions fall outside the recommended ranges, these conditions may be marginal and should not be changed in such a way as to make them more unfavorable.

Freshwater organisms

Dissolved Materials

(1) Dissolved materials that are relatively innocuous; i.e., their harmful effect is due to osmotic effects at high concentrations, should not be increased by more than one-third of the concentration that is characteristic of the natural condition of the subject water. In no instance should the concentration of total dissolved materials exceed 50 milliosmoles (the equivalent of 1500 mg/l NaCl).

(2) Dissolved materials that are harmful in relatively low concentrations are discussed in the section "Toxicity."

pH, Alkalinity, Acidity

(1) No highly dissociated materials should be added in quantities sufficient to lower the pH below 6.0 or to raise the pH above 9.0.

(2) To protect the carbonate system and thus the productivity of the water, acid should not be added in sufficient quantity to lower the total alkalinity to less than 20 mg/l.

(3) The addition of weakly dissociated acids and alkalies should be regulated in terms of their own toxicities as established by bioassay procedures.

Temperature

Warm Water Biota: To maintain a well-rounded population of warm-water fishes, the following restrictions on temperature extremes and temperature increases are recommended:

(1) During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water (at the expected minimum daily flow for that month) more than 5 F. In lakes, the temperature of the epilimnion in those areas where important organisms are most likely to be adversely affected should not be raised more than 3 F above that which existed before the addition of heat of artificial origin. The increase should be based on the monthly average of the maximum daily temperature. Unless a special study shows that a discharge

of a heated effluent into the hypolimnion will be desirable, such practice is not recommended and water for cooling should not be pumped from the hypolimnion to be discharged to the same body of water.

(2) The normal daily and seasonal temperature variations that were present before the addition of heat due to other than natural causes should be maintained.

(3) The recommended maximum temperatures that are not to be exceeded for various species of warm-water fish are given in table III-1.

Cold Water Biota: Because of the large number of trout and salmon waters which have been destroyed, made marginal, or nonproductive, remaining trout and salmon waters must be protected if this resource is to be preserved.

Inland trout streams, headwaters of salmon streams, trout and salmon lakes, and the hypolimnion of lakes and reservoirs containing salmonids and other cold water forms should not be warmed or used for cooling water. No heated effluents should be discharged in the vicinity of spawning areas.

For other types and reaches of cold-water streams, reservoirs and lakes, the following restrictions are recommended:

(1) During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water more than 5 F (based on the minimum expected flow for that month). In lakes, the temperature of the epilimnion should not be raised more than 3 F by the addition of heat of artificial origin.

(2) The normal daily and seasonal temperature fluctuations that existed before the addition of heat due to other than natural causes should be maintained.

TABLE III–1

[Provisional maximum temperatures recommended as compatible with the well-being of various species of fish and their associated biota]

93 F: Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, and gizzard shad.
90 F: Growth of largemouth bass, drum, bluegill, and crappie.
84 F: Growth of pike, perch, walleye, smallmouth bass, and sauger.
80 F: Spawning and egg development of catfish, buffalo, threadfin shad, and gizzard shad.
75 F: Spawning and egg development of largemouth bass, white and yellow bass, and spotted bass.
68 F: Growth or migration routes of salmonids and for egg development of perch and smallmouth bass.
55 F: Spawning and egg development of salmon and trout (other than lake trout).
48 F: Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon.

Note.—Recommended temperatures for other species, not listed above, may be established if and when necessary information becomes available.

(3) The recommended maximum temperatures that are not to be exceeded for various species of cold-water fish are given in table III–1.

Dissolved Oxygen

The following environmental conditions are considered essential for maintaining native populations of fish and other aquatic life.

(1) For a diversified warm-water biota, including game fish, DO concentration should be above 5 mg/l, assuming normal seasonal and daily variations are above this concentration. Under extreme conditions, however, they may range between 5 and 4 mg/l for short periods during any 24-hour period, provided that the water quality is favorable in all other respects. In stratified lakes, the DO requirements may not apply to the hypolimnion. In shallow unstratified lakes, they should apply to the entire circulation water mass.

These requirements should apply to all waters except administratively established mixing zones. In lakes, such zones must be restricted so as to limit the effect on the biota. In streams, there must be adequate and safe passageways for migrating forms. These must be extensive enough so that the majority of plankton and other drifting organisms are protected (see section on zones of passage).

(2) For the cold-water biota, it is desirable that DO concentrations be at or near saturation. This is especially important in spawning areas where DO levels must not be below 7 mg/l at any time. For good growth and the general well-being of trout, salmon, and their associated biota, DO concentrations should not be below 6 mg/l. Under extreme conditions, they may range between 6 and 5 mg/l for short periods provided the water quality is favorable in all other respects and normal daily and seasonal fluctuations occur. In large streams that have some stratification or that serve principally as migratory routes, DO levels may range between 4 and 5 mg/l for periods up to 6 hours, but should never be below 4 mg/l at any time or place.

(3) DO levels in the hypolimnion of oligotrophic small inland lakes and in large lakes should not be lowered below 6 mg/l at any time due to the addition of oxygen-demanding waste or other materials.

Carbon Dioxide

According to our present knowledge of the subject, it is recommended that the "free" carbon dioxide concentration should not exceed 25 mg/l.

Oil

Oil or petrochemicals should not be added in such quantities to the receiving waters that they will–

(1) produce a visible color film on the surface;
(2) impart an oily odor to the water or an oily or other noxious taste to fish and edible invertebrates;
(3) coat the banks and bottoms of the water course or taint any of the associated biota;
(4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity

(1) Turbidity in the receiving waters due to the discharge of wastes should not exceed 50 Jackson units in warm-water streams or 10 Jackson units in cold-water streams.

(2) There should be no discharge to warm-water lakes which would cause turbidities exceeding 25 Jackson units. The turbidity of cold-water or oligotrophic lakes should not exceed 10 units.

Settleable Materials

Since it is known that even minor deposits of settleable materials inhibit the growth of normal stream and lake flora, no such materials should be added to these waters in quantities that adversely affect the natural biota.

Color and Transparency

For effective photosynthetic production of oxygen, it is required that 10 percent of the incident light reach the bottom of any desired photosynthetic zone in which adequate dissolved oxygen concentrations are to be maintained.

Floating Materials

All floating materials of foreign origin should be excluded from streams and lakes.

Tainting Substance

All materials that will impart odor or taste to fish or edible invertebrates should be excluded from receiving waters at levels that produce tainting.

Radionuclides

(1) No radioactive materials should be present in natural waters as a consequence of the failure of an installation to exercise appropriate controls to minimize releases.

(2) No radionuclide or mixture of radionuclides should be present at concentrations greater than those specified by the USPHS Drinking Water Standards.

(3) The concentrations of radioactive materials present in fresh, estuarine, and marine waters should be less than those that would require restrictions on the use of organisms harvested from the area to meet the Radiation Protection Guides recommended by the Federal Radiation Council.

Plant Nutrients and Nuisance Growths

The Subcommittee wishes to stress that the concentrations set forth are suggested solely as guidelines and the maintenance of these may or may not prevent undesirable blooms. All the factors causing nuisance plant growth and the level of each which should not be exceeded are not known.

(1) In order to limit nuisance growths, the addition of all organic wastes such as sewage, food processing, cannery, and industrial wastes containing nutrients, vitamins, trace elements, and growth stimulants should be carefully controlled. Furthermore, it should be pointed out that the addition of sulfates or manganese oxide to a lake should be limited if iron is present in the hypolimnion as they may increase the quantity of available phosphorus.

(2) Nothing should be added that causes an increased zone of anaerobic decomposition of a lake or reservoir.

(3) The naturally occurring ratios and amounts of nitrogen (particularly NO_3 and NH_4) to total phosphorus should not be radically changed by the addition of materials. As a guideline, the concentration of total phosphorus should not be increased to levels exceeding 100μg/l in flowing streams or 50 μg/l where streams enter lakes or reservoirs.

(4) Because of our present limited knowledge of conditions promoting nuisance growth, we must have a biological monitoring program to determine the effectiveness of the control measures put into operation. A monitoring program can detect in their early stages the development of undesirable changes in amounts and kinds of rooted aquatics and the condition of algal growths. With periodic monitoring, such undesirable trends can be detected and corrected by more stringent regulation of added organics.

Toxic Substances

(1) **Substances of Unknown Toxicity:** All effluents containing foreign materials should be considered harmful and not permissible until bioassay tests have shown otherwise. It should be the obligation of the agency producing the effluent to demonstrate that it is harmless in the concentrations to be found in the receiving waters. All bioassays should be conducted strictly as recommended in the body of this report and the appropriate appli-

cation factor applied to determine the permissible concentration of toxicant.

(2) **Pesticides.**

(a) **Chlorinated hydrocarbons:** Any addition of chlorinated hydrocarbon insecticides is likely to cause damage to some desired organisms and should be avoided.

(b) **Other chemical pesticides:** Addition of other kinds of chemicals used as pesticides and herbicides can cause damage to desirable organisms and should be applied with utmost discretion and caution. Table III–5 (p. 62) lists the 48-hour TL_m values of a number of pesticides for various types of fresh water organisms. To provide reasonably safe concentrations of these materials in receiving waters, application factors ranging from $^1/_{10}$ to $^1/_{100}$ should be used with these values depending on the characteristic of the pesticide in question and used as specified in (4), below. Concentrations thus derived may be considered tentatively safe under the conditions specified.

(3) **Other Toxic Substances.**

(a) **ABS:** Concentration of continuous exposure to ABS should not exceed $^1/_7$ of the 48-hour TL_m. A concentration as high as 1 mg/l may be tolerated occasionally for periods of time not exceeding 24 hours. ABS may increase the toxicity of other materials.

(b) **LAS:** The concentration of LAS should not exceed 0.2 mg/l or $^1/_7$ of the 48-hour TL_m.

(4) **Application Factors:** Concentration of materials that are nonpersistant (that is, have a half-life of less than 96 hours) or have noncumulative effects after mixing with the receiving waters should not exceed $^1/_{10}$ of the 96-hour TL_m value at any time or place. The 24-hour average of the concentration of these materials should not exceed $^1/_{20}$ of the TL_m value after mixing. For other toxicants the concentrations should not exceed $^1/_{20}$ and $^1/_{100}$ of the TL_m value under the conditions described above. Where specific application factors have been determined, they will be used in all instances.

(5) **General Considerations.** When two or more toxic materials that have additive effects are present at the same time in the receiving water, some reduction is necessary in the permissible concentrations as derived from bioassays on individual substances or wastes. The amount of reduction required is a function of both the number of toxic materials present and their concentrations in respect to the derived permissible concentration. An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible concentration for the mixture is through the use of following relationship:

$$\left(\frac{C_a}{L_a}+\frac{C_b}{L_b}\;\ldots\;+\frac{C_n}{L_n}\leq 1\right)$$

Where C_a, C_b, . . . C_n are the measured concentrations of the several toxic materials in the water and L_a, L_b, . . . L_n are the respective permissible concentration limits derived for the materials on an individual basis. Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary.

Marine and estuarine organisms

Salinity

To protect estuarine organisms, no changes in channels, basin geometry, or freshwater influx should be made which would cause permanent changes in isohaline patterns of more than 10 percent of the naturally occurring variation.

Currents

Currents are important for transporting nutrients, larvae, and sedimentary materials for flushing and purifying wastes, and for maintaining patterns of scour and fill. To protect these functions, there should be no changes in basin geometry or freshwater inflow that will alter current patterns in such a way as to adversely affect existing biological and sedimentological situations.

pH

No materials that extend normal ranges of pH at any location by more than 0.1 pH unit should be introduced into salt water portions of tidal tributaries or coastal waters. At no time should the introduction of foreign materials cause the pH to be less than 6.7 nor greater than 8.5.

Temperature

In view of the requirements for the well-being and production of marine organisms, it is concluded that the discharge of any heated waste into any coastal or estuarine waters should be closely managed. Monthly means of the maximum daily temperatures recorded at the site in question and before the addition of any heat of artificial origin should not be raised by more than 4 F during the fall, winter, and spring (September through May), or by more than 1.5 F during the summer (June through August). North of Long Island and in the waters of the Pacific Northwest (north of California), summer limits apply July through September; and fall, winter, and spring limits apply October through June. The rate of temperature change should not exceed 1 F per hour except when due to natural phenomena.

Suggested temperatures are to prevail outside of established mixing zones as discussed in the section on zones of passage.

Dissolved Oxygen

Oxygen levels sufficient for the survival, growth, reproduction, general well-being, and production of a suitable crop must be maintained. The dissolved oxygen concentrations necessary to attain this objective in coastal waters, estuaries, and tidal tributaries are:

(1) Dissolved oxygen concentrations in surface coastal waters should be greater than 5.0 mg/l except when upwellings and other natural phenomena may cause this value to be depressed.

(2) Dissolved oxygen concentration in estuaries and tidal tributaries should not be less than 4.0 mg/l at any time or place except in naturally dystrophic waters or where natural conditions cause DO to be depressed.

Oil

No oil or petroleum products should be discharged into estuarine or coastal waters in quantities that: (1) Can be detected as a visible film, sheen, or by odor; (2) cause tainting of fish or edible invertebrates; (3) form an oil sludge deposit on the shores or bottom of the receiving body of water; (4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity

No effluent that may cause changes in turbidity or color should be allowed to enter estuarine or coastal waters unless it can be shown to have no deleterious effects on the aquatic biota.

Settleable and Floating Substances

No materials that contain settleable solids or substances that may precipitate out in quantities that adversely affect the biota should be introduced into coastal or estuarine waters. It is especially urgent that areas which serve as habitat or nursery grounds for commercially important species be protected from any impairment of natural conditions.

Tainting Substances

Substances that taint or produce off-flavors in fish and edible invertebrates should not be present in concentrations discernible by bioassay or organoleptic tests.

Radionuclides

The recommendations made for freshwater organisms apply to marine and estuarine organisms.

Plant Nutrients and Nuisance Organisms

(1) No changes should be made in the basin geometry, current structure, salinity, or temperature of the estuary until studies have shown that these changes will not adversely affect the biota or promote the increase of nuisance organisms.

(2) The artificial enrichment of the marine environment from all sources should not cause any major quantitative or qualitative alteration in the flora such as the production of persistant blooms of phytoplankton (whether toxic or not), dense growths of attached algae or higher aquatics, or any other sort of nuisance that can be attributed directly to nutrient excess or imbalance. Because these nutrients often are derived largely from drainage from land, special attention should be given to correct land management in river basins and shores of embayments to control unavoidable erosion.

(3) The naturally occurring atomic ratio of NO_3-N to PO_4-P in a body of water should be maintained. Similarly, the ratio of inorganic phosphorus (orthophosphate) to total phosphorus (the sum of inorganic phosphorus, dissolved organic phosphorus, and particulate phosphorus) should be maintained as it occurs naturally. Nutrient imbalances have been shown to cause a change in the natural diversity of desirable organisms and to reduce productivity.

Toxic Substances

(1) **Substances of Unknown Toxicity:** All effluents containing foreign materials should be considered harmful and not permissible until bioassay tests have shown otherwise. It should be the obligation of the agency producing the effluent to demonstrate that it is harmless in the concentrations that will be found in the receiving waters. All bioassays should be conducted strictly as recommended in the body of this report and the appropriate application factor applied to determine the permissible concentration of toxicant.

(2) **Pesticides for Which Limits Have Been Determined:** The pesticides are grouped according to their relative toxicity to shrimp. Criteria are based on the best estimates in the light of present knowledge and it is to be expected that acceptable levels of toxic materials may be changed as a result of future research.

Pesticide group A.—The following chemicals are acutely toxic at concentrations of 5 μg/l and less. On the assumption that $\frac{1}{100}$ of this level represents a reasonable application factor, it is recommended that environmental levels of these substances not be permitted to rise above 50 nanograms/l. This level is so low that these pesticides could not be applied directly in or near the marine habitat without danger of causing damage. The 48-hour TL_m is listed for each chemical in μg/l.

Organochloride pesticides

Aldrin	0.04	DDT	0.6
BHC	2.0	Dieldrin	0.3
Chlordane	2.0	Endosulfan	0.2
Endrin	0.2	Methoxychlor	4.0
Heptachlor	0.2	Perthane	3.0
Lindane	0.2	TDE	3.0
		Toxaphene	3.0

Organophosphorus pesticides

Coumaphos	2.0	Naled	3.0
Dursban	3.0	Parathion	1.0
Fenthion	0.03	Ronnel	5.0

Pesticide group B.—The following types of pesticide compounds are generally not acutely toxic at levels of 1.0 mg/l or less. It is recommended that an application factor of $\frac{1}{100}$ be used and in the absence of acute toxicity data that an environmental level of not more than 10 μg/l be permitted. An acute toxicity factor must be established for each specific chemical in this group to determine that it is not more toxic than related compounds as indicated above:

Arsenicals
Botanicals
Carbamates
2,4-D compounds
2,4,5-T compounds.
Phthalic acid compounds.
Triazine compounds.
Substituted urea compounds.

Other Pesticides.—Acute toxicity data are available for approximately 100 technical-grade pesticides in general use not listed in the above groups. These chemicals are either not likely to reach the marine environment or, if used as directed by the registered label, probably would not occur at levels toxic to marine biota. It is presumed that criteria established for these chemicals in fresh water will protect adequately the marine habitat. It should be emphasized that no unlisted chemical should be discharged into the estuary without preliminary bioassay tests.

(3) **Industrial and Other Toxic Wastes.**

(a) Safe concentrations of metals, ammonia, cyanide, and sulfide should be determined by the use of appropriate application factors to 96-hour TL_m values as determined by flow-through bioassays using dilution water that came from the receiving body. Test organisms should be local species or life stages of organisms of economic and ecologic importance which are the most sensitive to the waste in question. Application factors should be $\frac{1}{100}$ for metals, $\frac{1}{20}$ for ammonia, $\frac{1}{10}$ for cyanide, and $\frac{1}{20}$ for sulfide.

(b) Fluoride concentrations should not exceed those for drinking water.

(c) Permissible levels of detergents in fresh waters should also be applied to the marine and estuarine waters.

(d) Bacteriological criteria of estuarine waters utilized for shellfish cultivation and harvesting should conform with the standards as described in the National Shellfish Sanitation Program Manual of Operation. These standards provide that—

(*1*) examinations shall be conducted in accordance with the American Public Health Association recommended procedures for the examination of sea water and shellfish;

(*2*) there shall be no direct discharges of untreated sewage;

(*3*) samples of water for bacteriological examination to be collected under those conditions of time and tide which produce maximum concentration of bacteria;

(*4*) the coliform median MPN of the water does not exceed 70/100 ml, and not more than 10 percent of the samples ordinarily exceed an MPN of 230/100 ml for a five-tube decimal dilution test (or 330/100 ml, where the three-tube decimal dilution test is used) in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions;

(*5*) the reliability of nearby waste treatment plants shall be considered in the approval of areas for direct harvesting.

(e) Wastes from tar, gas, coke, petrochemical, pulp and paper manufacturing, waterfront and boating activities, hospitals, marine laboratories and research installation wastes are all complex mixtures having great variability in character and toxicity. Due to this variability, safe levels must be determined at frequent intervals by flow-through bioassays of the individual effluents.

For those operations having persistent toxicants, an application factor of $\frac{1}{100}$ should be used while for those composed largely of unstable or biodegradable toxicants, an application factor of $\frac{1}{20}$ is tentatively suggested.

(4) **General Considerations.**—When two or more toxicants that have additive effects are present, they must be treated as suggested earlier under fresh water organisms.

Wildlife

Dissolved Oxygen

In addition to the DO requirements for aquatic organisms, the bottoms of areas used by wildfowl must be kept aerobic to suppress botulinus organisms.

pH

Aquatic plants of greatest value as food for waterfowl thrive best in waters with a summer pH range of 7.0 to 9.2.

Alkalinity

Waterfowl habitats, to be productive, should have a bicarbonate alkalinity between 30 and 130 mg/l. Fluctuations should be less than 50 mg/l from natural conditions.

Salinity

Salinity should be kept as close to natural conditions as possible. Fluctuations in salinity during any 24-hour period should be limited as follows:

Natural salinity:	*Variation permitted*
0 to 3.5‰	1‰
3.5 to 13.5‰	2‰
13.5 to 35.0‰	4‰

Light Penetration

Optimum light requirements for aquatic wildlife habitats should be at least 10 percent of incident light at the surface to a 6-foot depth; the tolerable limit should be 5 percent of the light at the surface to the same depth.

Settleable Substances

Settleable substances destroy the usefulness of aquatic bottoms for waterfowl. Settleable substances should be excluded from areas expected to support waterfowl.

Oil

Oil is an especially dangerous substance to waterfowl. Oil and petrochemicals must be excluded from both the surface and bottoms of any area used by waterfowl.

Toxic Substances

Toxic substances should be excluded from wildlife habitats to the degree that they affect the health and well-being of wildlife, either directly or through biological magnification. Special consideration must be given to keep edible wildlife safe for consumption by humans.

Disease

Offal from poultry houses, meatpacking plants, as well as other possible sources of disease organisms, must be excluded from areas supporting wildlife to guard against transmission of such diseases as botulism, fowl cholera, and aspergillosis.

General

Water quality suitable for fish and other aquatic organisms will be adequate for wildlife.

fresh water organisms

Dissolved materials

Water devoid of dissolved materials is intolerable in nature because pure water will not support aquatic life. Natural waters contain endless varieties of dissolved materials in concentrations that differ widely from one locality to another as well as from time to time. Many of these dissolved materials are essential for growth, reproduction, and the general well-being of aquatic organisms. The chlorides, carbonates, and silicates of sodium, potassium, calcium, and magnesium are generally the most common salts present. Traces of most other essential substances are also found.

Aquatic organisms live in different concentrations of dissolved substances but productivity declines as the concentrations move away from the optimum. Seldom, if ever, are the dissolved substances at the optimum concentrations as we know them. The range of tolerance may be relatively wide, but when the concentrations reach too low or too high a level, organisms degenerate and die. Different organisms vary in their optimum requirements as well as in their ability to live and thrive under variations from the optimum. Some organisms are equally at home in sea water and in fresh water. Other organisms will tolerate only one or the other.

Any of the substances necessary to aquatic organisms has a range of concentration that is both essential and tolerable. The tolerance levels for any one substance vary depending on the concentrations of other substances present. The presence of certain substances synergizes the effects of some materials but antagonizes the effects of others. Under optimal concentrations, the synergistic and antagonistic effects are in balance and relatively high concentrations can be tolerated without adverse effects.

Although several measures of dissolved materials are available, no measure in itself is adequate as an index of optimum concentration nor is any single measure adequate to express the range of tolerance. The biological effects depend on the concentrations of the individual solutes, some of which are tolerated in terms of grams per liter but others only in nanograms per liter. Some exert considerable osmotic pressure, but for others the osmotic effect is negligible. Some substances contribute greatly to conductivity, while others have little or no effect.

In general, the concentrations of dissolved materials in natural fresh waters are below the optimum for maximum productivity. In many instances, therefore, the addition of any of a large number of substances will be beneficial. In this way, many water courses have a capacity to absorb materials to advantage. But the addition of what may be considered beneficial substances must be controlled so that they will not exceed favorable limits.

The osmotic concentration of the body fluids of a fresh water animal is generally the maximum concentration of dissolved material that the animal will tolerate. In some animals, notably some of the fresh water mollusks, the body fluids have an osmotic concentration as low as 50 milliosmoles (the equivalent of about 0.025 molar or 1,500 mg/l sodium chloride). If the dissolved materials are relatively innocuous, having only an osmotic effect, it is judged that the total dissolved materials in a water course may be increased to a certain extent but they should not exceed 50 milliosmoles if the fauna is to be maintained.

Many species of diatoms are very sensitive to changes in chloride and other salt concentrations. Some species, such as those in mountain streams and in black water streams of the coastal plains, can live only in waters with extremely low concentrations of salts. The addition of salts to such streams will eliminate many desirable species of diatoms and permit undesirable species to flourish. Such changes may reduce the desirable food sources and bring about nuisance problems as well. It is believed that the total dissolved material in a water course should not be increased by more than one-third of that which is characteristic of the natural conditions of such a water course.

The toxicity of substances added to natural waters often depends on the substances already present in the receiving waters. With synergism, the toxicity increases, and with antagonism it decreases. Again the reaction of the toxic substances may produce, in some cases, new products of greater toxicity, and in others, products of lesser toxicity.

In view of the many factors that become involved in the disposal of soluble materials in natural waters, it is evident that no simple answer is available. Therefore, bioassays should be used to determine the amounts of the materials that may be tolerated without reducing the productivity of the water course in question.

Recommendation: Dissolved materials are of two types: those that are toxic at very low concentrations and those, such as the salts of the earth metals, that are required in certain concentrations for a productive water and become harmful only at high concentrations by exerting an osmotic effect. If the dissolved materials are relatively innocuous, i.e., their harmful effect is an osmotic one at high concentrations, it is judged that the total dissolved materials of this type may be increased to a certain extent but they should not exceed 50 milliosmoles in waters where diversified animal populations are to be protected. Further, to maintain local conditions, total dissolved materials should not be increased by more than one-third of the concentration that is characteristic of the natural condition of the water. When dissolved materials are being increased, bioassays and field studies should be used to determine how much of the materials may be tolerated without reducing the productivity of the desired organisms.

Acidity alkalinity, and pH

Acidity and alkalinity are reciprocal terms. Acidity is produced by substances that yield hydrogen ions on hydrolysis and alkalinity is produced by substances that yield hydroxyl ions. Other definitions state that a substance is acid if it will neutralize hydroxyl ions and a substance is alkaline if it will neutralize hydrogen ions. The terms "total acidity" and "total alkalinity" are often used to express the buffering capacity of a solution. Acidity in natural waters is caused by carbon dioxide, mineral acids, weakly dissociated acids, and the salts of strong acids and weak bases. Alkalinity is caused by strong bases and the salts of strong alkalies and weak acids.

An index of the hydrogen ion activity is pH. Even though pH determinations are used as an indication of acidity and/or alkalinity, pH is not a measure of either. As pointed out in the first sentence in the previous paragraph, acidity and alkalinity are reciprocal terms. Indeed, a water may have both an acidity and alkalinity at the same time. Total acidity, by definition, is the amount of standard alkali required to bring a sample to pH 8.3. Total alkalinity, similarly, is the amount of standard acid required to bring a sample to pH 4.5. Both are expressed in equivalents of $CaCO_3$. Under these circumstances, there is a relationship between pH, acidity, and alkalinity since, by definition (see Standard Methods for the Examination of Water and Wastewater, 12th edition, 1965), any water with a pH of 4.5 or lower has no measurable alkalinity and a water with a pH of 8.3 or higher has no measurable acidity.

In natural waters, where the pH is in the vicinity of 8.3, acidity is not a factor of concern. In most productive, fresh, natural waters, the pH falls in the range between 6.5 and 8.5 (except when increased by photosynthetic activity). Some aquatic organisms have been found to live at pH 2 and lower and others at pH 10 and higher; however, such organisms are relatively few. Some natural waters with a pH of 4 support fish and other organisms. In these cases the acidity is due primarily to carbon dioxide and humic acids and the water has little buffering capacity (low total alkalinity). Other natural waters with a pH of 9.5 also support fish, but in such situations the waters are not regarded as highly productive.

Acids that dissociate to a high degree do not appear to be toxic at pH values above 6.0. They are toxic if added in sufficient quantities to reduce the pH to less than 6.0 Acids that dissociate to a low degree are often toxic at pH values considerably above 6.0. In the latter condition, toxicity is due either to the anion or to the compound itself; e.g., hydrogen cyanide (HCN), hydrogen sulfide (H_2S), and hypochlorous ($HClO$) and tannic acids.

Alkalies that dissociate to a high degree do not appear to be toxic at pH values below 9.0. Alkaline compounds that dissociate to a low degree are often toxic at pH values less than 9.0 and their toxicity is due either to the cation or to the undissociated molecule. Ammonium hydroxide is an example. Temporarily high pH levels often are produced in highly productive waters through photosynthetic activity of the aquatic plants by converting the carbonate to the hydroxide, which results in an increased pH. Because these high pH levels prevail for only a few hours, they do not produce the harmful effects of continuous high levels due to the presence of strong alkalies.

Addition of either acids or alkalies to waters may be harmful not only in producing adverse acid or alkaline conditions, but also by increasing the toxicity of various components in the waters. The addition of strong acids may cause the formation of carbonic acid (free CO_2) in quantities that are adverse to the well-being of the organisms present. A reduction of about 1.5 pH units can cause a thousand-fold increase in the acute toxicity of a metallo-cyanide complex. The addition of strong alkalies may cause the formation of undissociated NH_4OH or un-ionized NH_3 in quantities that may be toxic. The availability of many nutrient substances varies with the acidity and alkalinity. At higher pH values, iron tends to become unavailable to some plants.

The nonlethal limits of pH are narrower for some fish food organisms than they are for fish. For example, *Daphnia magna* does not survive experimentally in water having a pH below 6.0.

The major buffering system in natural waters is the carbonate system. This system not only neutralizes acids and bases so as to reduce the fluctuations in pH, but also forms an indispensable reservoir of carbon for photosynthesis, because there is a decided limit on the rate at which carbon dioxide can be obtained from the atmosphere to replace that in the water which becomes fixed by the plants. Thus the productivities of waters are closely correlated with the carbonate buffering systems. The addition of mineral acids preempts the carbonate buffering capacity and the original biological productivity is reduced in proportion to the degree that such capacity is exhausted. It is as necessary, therefore, to maintain the minimum essential buffering capacity as it is to confine the pH of the water within tolerable limits.

Recommendation: (1) In view of the above considerations and their importance for the production and well-being of aquatic organisms, no highly dissociated materials should be added in quantities sufficient to lower the pH below 6.0 or to raise the pH above 9.0.

(2) To protect the carbonate system and thus the productivity of the water, acid should not be added sufficient to lower the total alkalinity below 20 mg/l expressed as $CaCO_3$.

(3) The addition of weakly dissociated acids and alkalies should be regulated in terms of their own toxicities as established by bioassay procedures.

Hardness

Hardness was originally considered as the capacity of water to precipitate or neutralize soap. In natural waters, hardness is chiefly attributable to calcium and magnesium ions. Other ions, such as strontium, barium, aluminum, manganese, iron, copper, zinc, and lead also are responsible for hardness, but since they are present in relatively minor concentrations, their role usually can be ignored. Hardness, like acidity and alkalinity, is expressed in terms of $CaCO_3$ but the hardness of a water is not necessarily equal to either the acidity or alkalinity. Hardness in natural waters is generally correlated with dissolved solids but there are exceptions.

Generally, the biological productivity of a water is directly correlated with its hardness, but hardness *per se* has no biological significance because productivity depends on the specific combination of elements present. Calcium and magnesium contribute to hardness and to productivity. Most other elements that contribute to hardness reduce biological productivity and are toxic when they produce a substantial measure of hardness. Because hardness of itself has no biological significance, and because some elements which contribute to hardness may enhance biological productivity (while other contributing elements are toxic), it is recommended that the term hardness be avoided in dealing with water quality requirements for aquatic life.

Temperature

The relationships of temperature and aquatic life have been well studied. Extensive bibliographies and detailed surveys of the subject have been published by the American Society of Civil Engineers (1967), Brett (1960), Mihursky and Kennedy (1967), Raney (1966), U.S. Department of Interior, Federal Water Pollution Control Administration (1967), and Wurtz and Renn (1965).

The temperatures of the surface waters of the United States vary from 32 to over 100 F as a function of latitude, altitude, season, time of day, duration of flow, depth, and many other variables. The agents that may affect the natural temperature are so numerous that it seems unlikely that two bodies of water, even in the same latitude, would have exactly the same thermal characteristics. The fish and other aquatic life occurring naturally in each body of water are species or varieties that are competing there with various degrees of success depending on the temperature and various other conditions existing in that habitat. This adaptation extends not only to temperature and the range over which it can vary, but also to such factors as day length and the other species of animals and plants in the same habitat. The interrelationships of species, day length, and water temperature are so intimate that even a small change in temperature may have far-reaching effects. An insect nymph in an artificially warmed stream, for example, might emerge for its mating flight too early in the spring and be immobilized by the air temperature. Similarly, a fish might hatch too early in the spring to find an adequate amount of its natural food organisms because the food chain depends ultimately on plants whose abundance in turn, is a function of day length and temperature. The inhabitants of a water body that seldom becomes warmer than 70 F are placed under stress, if not killed outright, by 90 F water. Even at 75 to 80 F, they may be unable to compete successfully with organisms for which 75 to 80 F is a favorable temperature. Similarly, the inhabitants of warmer waters are at a competitive disadvantage in cool water.

Although in a rigorous climate, an animal can endure the extremes of temperature at appropriate seasons; it must be cooled gradually in the fall if it is to become acclimatized to the cold water of winter and warmed gradually in the spring if it is to withstand summer heat. Further, an organism might be able to endure a high temperature of 92 or 95 F for a few hours, but it could not do so for a period of days. Having the water change gradually with the season is important for other reasons: an increasing or decreasing temperature often serves as the trigger for spawning activities, metamorphosis, and migration. Some fresh water organisms require that their eggs be chilled before they will hatch properly.

In arriving at suitable temperature criteria, the problem is to estimate how far the natural temperature may be exceeded without adverse effects. Whatever requirements are suggested, a seasonal cycle must be retained, the changes in temperature must be gradual and the temperature reached must not be so high or so low as to damage or alter the composition of the desired population. In view of the many variables, it seems obvious that no single temperature requirement can be applied to the United States as a whole, or even to one State; the requirements must be closely related to each body of water and its population. To do this a temperature increment based on the natural water temperature is more appropriate than an unvarying number. Using an increment requires, however, that we have information on the natural temperature conditions of the water in question, and the size of the increment that can be tolerated by the desired species.

If any appreciable heat load is introduced into a stream, it must be recognized that the species' equilibrium will likely be shifted towards that characteristic of a more southerly water.

The seasonal temperature fluctuation normal to the desired biota of a particular water must be maintained. Further, the sum of any increase in temperature plus the natural peak temperature should be of short duration and below the maximum temperature that is detrimental for such periods.

Recommendation for Warm Waters: To maintain a well-rounded population of warm-water fishes, the following restrictions on temperature extremes and temperature increases are recommended:

(1) During any month of the year, heat should not be added to a stream in excess of the amount that will raise the temperature of the water (at the expected minimum daily flow for that month) more than 5 F. In lakes and reservoirs, the temperatures of the epilimnion, in those areas where important organisms are most likely to be adversely affected, should not be raised more than 3 F above that which existed before the addition of heat of artificial origin. The increase should be based on the monthly average of the maximum daily temperature. Unless a special study shows that a discharge of a heated effluent into the hypolimnion or pumping water from the hypolimnion (for discharging back into the same water body) will be desirable, such practice is not recommended.

(2) The normal daily and seasonal temperature variations that were present before the addition of heat, due to other than natural causes, should be maintained.

(3) The recommended maximum temperatures that are not to be exceeded for various species of warm-water fish are given in table III-1.

Recommendation for Cold Waters: Because of the large number of trout and salmon waters which have been destroyed, or made marginal or nonproductive, the remaining trout and salmon waters must be protected if this resource is to be preserved:

(1) Inland trout streams, headwaters of salmon streams, trout and salmon lakes and reservoirs, and the hypolimnion of lakes and reservoirs containing salmonids should not be warmed. No heated effluents should be discharged in the vicinity of spawning areas.

For other types and reaches of cold-water streams, reservoirs, and lakes, the following restrictions are recommended.

(2) During any month of the year, heat should not be added to a stream in excess of the amount that will raise the temperature of the water more than 5 F (based on the minimum expected flow for that month). In lakes and reservoirs, the temperature of the epilimnion should not be raised more than 3 F by the addition of heat of artificial origin.

(3) The normal daily and seasonal temperature fluctuations that existed before the addition of heat due to other than natural causes should be maintained.

(4) The recommended maximum temperatures that are not to be exceeded for various species of cold water fish are given in table III–1.

NOTE.—For streams, total added heat (in BTU's) might be specified as an allowable increase in temperature of the minimum daily flow expected for the month or period in question. This would allow addition of a constant amount of heat throughout the period. Approached in this way for all periods of the year, seasonal variation would be maintained. For lakes the situation is more complex and cannot be specified in simple terms.

TABLE III–1

[Provisional maximum temperatures recommended as compatible with the well-being of various species of fish and their associated biota]

93 F: Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, and gizzard shad.

90 F: Growth of largemouth bass, drum, bluegill, and crappie.

84 F: Growth of pike, perch, walleye, smallmouth bass, and sauger.

80 F: Spawning and egg development of catfish, buffalo, threadfin shad, and gizzard shad.

75 F: Spawning and egg development of largemouth bass, white, yellow, and spotted bass.

68 F: Growth or migration routes of salmonids and for egg development of perch and smallmouth bass.

55 F: Spawning and egg development of salmon and trout (other than lake trout).

48 F: Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon.

Note.—Recommended temperatures for other species, not listed above, may be established if and when necessary information becomes available.

Dissolved oxygen

Oxygen requirements of aquatic life have been extensively studied. Excellent survey papers are presented by Doudoroff (1957), Doudoroff and Shumway (1967), Doudoroff and Warren (1962), Ellis (1937), and Fry (1960). Much of the work on temperature requirements also considers oxygen and those bibliographies are equally valuable.

Most of the research concerning oxygen requirements for freshwater organisms deals with fish, but since fish depend upon other aquatic species for food and would not remain in an area with an inadequate food supply, it seems reasonable to assume that a requirement for fish would serve also for the rest of the community. The fish themselves can be grouped into three categories according to their temperature and oxygen requirements: (1) the cold-water fish (e.g., salmon and trout), (2) the warm-water game and pan fish (e.g., bass and sunfish), and (3) the warm-water "coarse" fish (e.g., carp and buffalo). The cold-water fish seem to require higher oxygen concentrations than the warm-water varieties. The reason is not known, but it may be related to the fact that, for half saturation, trout hemoglobin requires an oxygen partial pressure three or four times that required by carp hemoglobin under similar circumstances. Warm-water game and pan fish seem to require a higher concentration than the "coarse" fish, probably because the former are more active and predatory.

Relatively little of the research on the oxygen requirements of fish in any of these three categories is applicable to the problem of establishing oxygen criteria because the endpoints have usually been too crude. It is useless in the present context to know how long an animal can resist death by asphyxiation at low dissolved oxygen concentrations; we must know instead the oxygen concentration that will permit an aquatic population to thrive. We need data on the oxygen requirements for egg development, for newly hatched larvae, for normal growth and activity, and for completing all stages of the reproductive cycle. It is only recently that experimental work has been undertaken on the effects of oxygen concentration on these more subtle endpoints. As yet, only a few species have been studied.

One of the first signs that a fish is being affected by a reduction of dissolved oxygen (DO) concentration is an increase in the rate at which it ventilates its gills, a process accomplished in part by an increase in the frequency of the opercular movements. The half dozen or so species (chiefly warm-water game and pan fish) that have been reported so far show a significant increase in frequency as the DO concentration is reduced from 6 to 5 mg/l (at about 72 F) and a greater increase from 5 to 4 mg/l. If the opercular rate is taken as the criterion by which the adequacy of an oxygen concentration is to be judged, then such evidence as we have indicates 6 mg/l as the required dissolved oxygen concentration. Several field studies have shown, however, that good and diversified fish populations can occur in waters in which the dissolved oxygen concentration is between 6 and 5 mg/l in the summer, suggesting that a minimum of 6 mg/l is probably more stringent than necessary for warm-water fishes. Because the oxygen content of a body of water does not remain constant, it follows that if the dissolved oxygen is never less than 5 mg/l it must be higher part of the time. In some cases, good populations of warm-water fish, including game and pan fishes, occur in waters in which the dissolved oxygen may be as low as 4 mg/l for short periods. Three mg/l is much too low, however, if normal growth and activity are to be maintained. It has been reported that the growth of young fish is slowed markedly if the oxygen concentration falls to 3 mg/l for part of the day, even if it rises as high as 18 mg/l at other times. It is for such reasons as this that oxygen criteria cannot be based on averages. Five and 4 mg/l are close to the borderline of oxygen concentrations that are tolerable for extended periods. For a good population of game and pan fishes, the concentration should be considerably more than this.

The requirements of the different stages in the life cycles of aquatic organisms must be taken into account. An oxygen concentration that can be tolerated by an adult animal, with fully developed respiratory apparatus, less intense metabolic requirements, and the ability to move away from adverse conditions, could easily be too low for eggs and larval stages. The eggs are especially vulnerable to oxygen lack because they have to depend upon oxygen diffusing into them at a rate sufficient to maintain the developing embryos. Hatching, too, is a critical time; recently hatched young need relatively more oxygen than adults, but until they become able to swim for themselves (unless they are in flowing water) they must depend upon the oxygen supply in the limited zone around them. These problems are not as great among species that tend their eggs and young, suspend their eggs from plants, or have pelagic eggs, as they are for salmonids. Salmonids bury their eggs in the gravel of the stream away from the main flow of the water thereby requiring a relatively high oxygen concentration in the water that does reach them.

Recommendation: In view of the above considerations and with the proviso that future research may make revision necessary, the following environmental conditions are considered essential for maintaining native populations of fish and other aquatic life:

(1) For a diversified warm-water biota, including game fish, daily DO concentration should be above 5 mg/l, assuming that there are normal seasonal and daily variations above this concentration. Under extreme conditions, however, and with the same stipulation for seasonal and daily fluctuations, the DO may range between 5 mg/l and 4 mg/l for short periods of time, provided that the water quality is favorable in all other respects. In stratified eutrophic and dystrophic lakes, the DO requirements may not apply to the hypolimnion. In shallow unstratified lakes, they should apply to the entire circulating water mass.

These requirements should apply to all waters except administratively established mixing zones. In lakes, such mixing zones must be restricted so as to limit the effect on the biota. In streams, there must be no blocks to migration and there must be adequate and safe passageways for migrating forms. These zones of passage must be extensive enough so that the majority of plankton and other drifting organisms are protected (see section on zones of passage).

(2) For the cold water biota, it is desirable that DO concentrations be at or near saturation. This is especially important in spawning areas where DO levels must not be below 7 mg/l at any time. For good growth and the general well-being of trout, salmon, and other species of the biota, DO concentrations should not be below 6 mg/l. Under extreme conditions they may range between 6 and 5 mg/l for short periods provided that the water quality is favorable and normal daily and seasonal fluctuations occur. In large streams that have some stratification or that serve principally as migratory routes, DO levels may be as low as 5 mg/l for periods up to 6 hours, but should never be below 4 mg/l at any time or place.

(3) DO levels in the hypolimnion of oligotrophic small inland lakes and in large lakes should not be lowered below 6 mg/l at any time due to the addition of oxygen-demanding wastes or other materials.

Carbon dioxide

An excess of "free" carbon dioxide (as distinguished from that present as carbonate and bicarbonate) may have adverse effects on aquatic animals. These effects range from avoidance reactions and changes in respiratory movements at low concentrations, through interference with gas exchange at higher concentrations, to narcosis and death if the concentration is increased further. The respiratory effects seem the most likely to be of concern in the present connection.

Since the carbon dioxide resulting from metabolic processes leaves the organisms by diffusion, an increase in external CO_2 concentration will make it more difficult for it to diffuse out of the organism. Thus, it begins to accumulate internally. The consequences of this internal accumulation are best known for fish, but presumably the principles are the same for other organisms. As the CO_2 accumulates, it depresses the blood pH, and this may have detrimental effects. Probably more important, however, is the fact that the greater the blood CO_2 concentration, the less readily will the animal's hemoglobin combine with dissolved oxygen. Thus the presence of much CO_2 raises the minimum oxygen concentration which is tolerable. Since the combination of oxygen with hemoglobin is inversely related to temperature, it is obvious that CO_2, temperature, and oxygen are closely related. Insufficient data are available at present to permit us to state the greatest amount of dissolved carbon dioxide that all types of aquatic organisms can tolerate and how these tolerable concentrations vary with temperature and dissolved oxygen. Studies of the effect of CO_2 on the oxygen requirements of several species of fish indicate that CO_2 concentrations of the order of 25 mg/l should not be detrimental, provided the oxygen concentration and temperature are within the recommended limits.

Recommendation: According to our rather meagre knowledge of the subject, it is recommended that the free CO_2 concentration should not exceed 25 mg/l.

Oil

Oil slicks are barely visible at a concentration of about 25 gal/sq mi (Amer. Petroleum Inst. 1949). At 50 gal/sq mi, an oil film is 3.0×10^{-6} inches thick and is visible as a silvery sheen on the surface. Sources of oil pollution are bilge and ballast waters from ships, oil refinery wastes, industrial plant wastes such as oil, grease, and fats from the lubrication of machinery, reduction works, plants manufacturing hydrogenated glycerides, free fatty acids, and glycerine, rolling mills, county drains, storm-water overflows, gasoline filling stations, and bulk stations.

Wiebe (1935) showed that direct contact by fish (bass and bream) with crude oil resulted in death caused by a film over the gill filaments. He also demonstrated that crude oil contains a water-soluble fraction that is very toxic to fish. Galtsoff, et al. (1935) showed that crude oil contains substances soluble in sea water that produce an anaesthetic effect on the ciliated epithelium of the gills of oysters. Free oil and emulsions may act on the epithelial surfaces of fish gills and interfere with respiration. They may coat and destroy algae and other plankton, thereby removing a source of fish food, and when ingested by fish they may taint their flesh.

Setteable oily substances may coat the bottom, destroy benthic organisms, and interfere with spawning areas. Oil may be absorbed quickly by suspended matter, such as clay, and then due to wind action or strong currents may be transported over wide areas and deposited on the bottom far from the source. Even when deposited on the bottom, oil continuously yields water-soluble substances that are toxic to aquatic life.

Films of oil on the surface may interfere with reaeration and photosynthesis and prevent the respiration of aquatic insects such as water boatmen, backswimmers, the larvae and adults of many species of aquatic beetles, and some species of aquatic Diptera (flies). These insects surface and carry oxygen bubbles beneath the surface by means of special setae which can be adversely affected by oil. Berry (1951) reported that oil films on the lower Detroit River are a constant threat to waterfowl. Oil is detrimental to waterfowl by destroying the natural buoyancy and insulation of their feathers.

A number of observations made by various authors in this country and abroad record the concentrations of oil in fresh water which are deleterious to different species. For instance, penetration of motor oil into a fresh water reservoir used for holding crayfish in Germany caused the death of about 20,000 animals (Seydell, 1913). It was established experimentally that crayfish weighing from 35 to 38 g die in concentrations of 5 to 50 mg/l within 18 to 60 hours. Tests with two species of fresh water fish, ruff (small European perch), and whitefish (fam. Coregonidae) showed that concentrations of 4 to 16 mg/l are lethal to these species in 18 to 60 hours.

The toxicity of crude oil from various oil fields in Russia varies depending on its chemical composition. The oil used by Veselov (1948) in the studies of the pollution of Belaya River (a tributary in the Kama in European Russia) belongs to a group of methano-aromatic oils with a high content of asphalt, tar compounds, and sulfur. It contains little paraffin and considerable amounts of benzene-ligroin. Small crucian carp (*Carassius carassius*) 7–9 cm long were used as the bioassay test animal. This is considered to be a hardy fish that easily withstands adverse conditions. The water soluble fraction of oil was extracted by shaking 15 ml of oil in 1 liter of water for 15 minutes. The oil film was removed by filtration. Dissolved oxygen was controlled. A total of 154 tests were performed using 242 fishes. The average survival time was 17 days at the concentration of 0.4 ml/l of oil but only 3 days at the concentration of 4 ml/l. Further increase in concentration had no appreciable effect on fish mortality.

Seydell (1913) stated that the toxicity of Russian oil is due to naphthenic acids, small quantities of phenol, and volatile acids (Veselov, 1948).

Cairns (1957) reports the following 96-hour TL$_m$ values of naphthenic acid for bluegill sunfish (*Lepomis macrochirus*)—5.6 mg/l; pulmonate snail (*Physa heterostropha*)—6.1 to 7.5 mg/l (in soft water), and diatom (species not identified)—41.8 to 43.4 mg/l in soft water and 28.2 to 79.8 mg/l in hard water. Naphthenic acid (cyclohexane carboxylic acid) is extracted from petroleum and is used in the manufacture of insecticides, paper, and rubber.

Chipman and Galtsoff (1949) report that crude oil in concentrations as low as 0.3 mg/l is extremely toxic to fresh water fish. Dorris, Gould, and Jenkins (1960) made an intensive study of the toxicity of oil refinery effluents to fathead minnows in Oklahoma. By standard bioassay procedures, they found that mortality varied between 3.1 percent to 21.5 percent after 48 hours of exposure to untreated effluents. They concluded that toxicity rather than oxygen demand is the most important effect of oil refinery effluents on receiving streams.

Pickering and Henderson (1966b) reported the results of acute toxicity studies of several important petrochemicals to fathead minnows, bluegills, goldfish, and guppies in both soft water and hard water. Standard bioassay methods were used. Because several of the compounds tested have low solubility in water, stock solutions were prepared by blending the calculated concentrations into 500 ml of water before addition to the test container. Where necessary, pure oxygen was supplied by bubbling at a slow rate. The petrochemicals tested were benzene, chlorobenzene, 0-chlorophenol, 3-chloropropene, 0-cresol, cyclohexane, ethyl benzene, isoprene, methyl methacrylate, phenol, 3-phthalic anhydride, styrene, toluene, vinyl acetate, and xylene. These petrochemicals are similar in their toxicities to fish, with 96-hour TL$_m$ values ranging from 12 to 368 mg/l. Except for isoprene and methyl methacrylate, which are less toxic, values for all four species of fish for the other petrochemicals ranged from 12 to 97 mg/l, a relatively small variation. In general, 0-chlorophenol and 0-cresol are the most toxic and methyl methacrylate and isoprene are the least toxic.

Recommendation: In view of available data, it is concluded that to provide suitable conditions for aquatic life, oil and petrochemicals should not be added in such quantities to the receiving waters that they will: (1) produce a visible color film on the surface, (2) impart an oily odor to water or an oily taste to fish and edible invertebrates, (3) coat the banks and bottom of the water course or taint any of the associated biota, or (4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity

Turbidity is caused by the presence of suspended matter such as clay, silt, finely divided organic matter, bacteria, plankton, and other microscopic oragnisms. Turbidity is an expression of the optical property of a sample of water which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. Excessive turbidity reduces light penetration into the water and, therefore, reduces photosynthesis by phytoplankton organisms, attached algae, and submersed vegetation.

The Jackson candle turbidimeter (Standard Methods for the Examination of Water and Wastewater, 12th edition, 1965) is the standard instrument for making measurements of turbidity. Field determinations, however, are made with direct-reading colorimeters calibrated for this test and the results are expressed as Jackson turbidity units (JTU).

Silt and sediment are particularly damaging to gravel and rubble-type bottoms. The sediment fills the interstices between gravel and stones, thereby eliminating the spawning grounds of fish and the habitat of many aquatic insects and other invertebrate animals such as mollusks, crayfish, fresh water shrimp, etc. Tarzwell (1957) observed that bottom organisms from a silted area averaged only 36 organisms/sq ft compared to 249/sq ft in a non-silted area. Smith (1940) reported that silting reduced the bottom fauna of the Rogue River by 25 to 50 percent. Observations in Oregon by Wagner (1959) and Ziebell (1960) showed an 85-percent decline in productivity of aquatic insect populations below a gravel dragline operation. Turbidities in the affected area were increased from zero to 91 mg/l and suspended solids from 2 mg/l upstream to 103 mg/l downstream.

Buck (1956) investigated several farm ponds, hatchery ponds, and reservoirs over a 2-year period. He observed that the maximum production of 161.5 lb/acre occurred in farm ponds where the average turbidity was less than 25 JTU. Between 25 and 100 JTU, fish yield dropped 41.7 percent to 94 lb/acre, and in muddy ponds, where turbidity exceeded 100 JTU, the yield was only 29.3 lb/acre or 18.2 percent of clear ponds.

Herbert and Merkens (1961), using a mixture of kaolin and diatomaceous earth, demonstrated that long-term exposure of rainbow trout to 100–200 mg/l could be harmful. At 270 and 810 mg/l, a high percentage of the fish died. Wallen (1951) studied the effects of montmorillonite clay on 16 species of warm-water fish. Results are shown in table III–2. It is shown that fish can tolerate high turbidities for short periods, a fortunate adaptation for river species. Fish productivity is ultimately dependent upon plant life and a good bottom fauna. There can be little of either above 200 JTU if that turbidity is maintained continuously. The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (ORSANCO) Second Progress Report (1956) points out that fish withstand turbidities of 5,000 mg/l or more with no direct harmful results, but the productivity of the bottom areas is very low and the fish populations are small.

TABLE III–2. Average Turbidities Found To Be Fatal to Fish

Species	Length of exposure (days)	Turbidity (mg/l)
Large mouth bass	7.6	101,000
Pumpkin seed sunfish	13	69,000
Channel catfish	9.3	85,000
Black bullhead	17	222,000
Golden shiner	7.1	166,000

Ellis (1937) summarized the results of 2,344 light penetration determinations made at 585 stations on streams throughout the United States. The determinations were made of the millionth intensity depth (m.i.d.), which is the depth in millimeters of water of the given turbidity required to screen out 99.9999 percent of the light entering at the surface. A photoelectric apparatus described by Ellis (1934b) was used and determinations were made after filtering the water through bolting silk.

The turbidity of rivers varies widely in different parts of the country. Ellis (1937) defined clear streams as those with a m.i.d. of 5.00 to infinity; cloudy streams, 4.90–1.00 meters; turbid, 0.99–0.50; very turbid, 0.49–0.30; muddy, 0.29–0.15; very muddy, 0.14–0.00 meters.

In Mississippi River side channels and flowing stream tributaries with good fish fauna, 4 percent were clear, 11 percent cloudy, 3 percent were very muddy. In these waters, with medium, poor, or no fish fauna 1 percent were clear, 18 percent cloudy, 11 percent turbid, 14 percent very turbid, 38 percent muddy, and 18 percent very muddy.

Based on 6,000 light penetration determinations on inland streams, he concluded that, for good production of fish and aquatic life, the silt load of these streams should be reduced so that the millionth intensity depth would be greater than 5 meters.

Good farming practices can do a great deal to prevent silt from reaching streams and lakes. Road building and housing development projects, placer mining, strip mining, coal and gravel washing, and unprotected road cuts are important sources of turbidity that can be reduced with planning, good housekeeping, and regulation.

Natural turbidities within watersheds should be determined. For example, in some Western States many streams have a turbidity below 25 JTU for most of the year. In those states, the water pollution control agency might specify that no wastes should be discharged which would raise the turbidity of the receiving water above 25 JTU.

From the above discussion it can be seen that natural turbidity varies greatly in different parts of the country.

Recommendation: Turbidity in the receiving water due to a discharge should not exceed 50 JTU in warm-water streams or 10 JTU in cold-water streams.

There should be no discharge to warm-water lakes which will cause turbidities exceeding 25 Jackson Units. The turbidity of cold-water or oligotrophic lakes should not exceed 10 units.

Settleable solids

Settleable solids include both inorganic and organic materials. The inorganic components include sand, silt, and clay originating from such sources as erosion, placer mining, mine tailing wastes, strip mining, gravel washing, dusts from coal washeries, loose soils from freshly plowed farm lands, highway, and building projects. The organic fraction includes such settleable materials as greases, oils, tars, animal and vegetable fats, paper mill fibers, synthetic plastic fibers, sawdust, hair, greases from tanneries, and various settleable materials from city sewers. These solids may settle out rapidly and bottom deposits are often a mixture of both inorganic and organic solids. They may adversely affect fisheries by covering the bottom of the stream or lake with a blanket of material that destroys the bottom fauna or the spawning grounds of fish. Deposits containing organic materials may deplete bottom oxygen supplies and produce hydrogen sulfide, carbon dioxide, methane, or other noxious gases.

Some settleable solids may cause damage by mechanical action.

Water Quality Criteria for European Freshwater Fish (European Inland Fisheries Advisory Commission, 1964) discusses chemically inert solids in waters that are otherwise satisfactory for the maintenance of freshwater fisheries. It is indicated that good or moderate fisheries can be maintained in waters that normally contain 25 to 80 mg/l suspended solids, but that the yield of fish might be lower than in waters containing 25 mg/l or less. Waters normally containing 80 to 400 mg/l suspended solids are unlikely to support good freshwater fisheries.

Recommendation: Since it is known that even minor deposits of settleable materials inhibit the growth of normal stream or lake flora and fauna, it is recommended that no settleable materials be added to these waters in quantities that adversely affect the natural biota.

Color

The color of water is attributed to substances in solution after the suspensoids have been removed. It may be of organic or mineral origin. Organic sources are humic materials, peat, plankton, rooted and floating aquatic plants, tannins, etc. Inorganic sources are metallic substances such as iron and manganese compounds and chemicals, dyes, etc. Many industries discharge materials that contribute to the color of water. Among them are pulp and paper mills, textile mills, refineries, manufacturers of chemicals and dyes, explosives, nailworks, tanneries, etc.

Standard Methods for the Examination of Water and Wastewater, 12th edition (1965), describes the standard platinum-cobalt method of determining color after centrifugation. The unit of color considered as standard is the color produced by one mg/l of platinum in water. Results are expressed as units of color. Color in excess of 50 units may limit photosynthesis and have a deleterious effect upon aquatic life, particularly phytoplankton, and the benthos.

Water absorbs light differentially. A layer of distilled water 1 meter in thickness absorbs 53 percent of the solar radiation. It absorbs 30 percent of the red-orange band (6,500 angstrom units) but less than 5 percent of the blue (4,500 angstrom units). These are the portions of the spectrum that are absorbed and utilized to the greatest extent by chlorophyll. The band at 7,500 angstrom units is over 90 percent absorbed.

Natural waters absorb far more light. The light intensity at which the amount of oxygen produced photosynthetically is balanced by the amount of oxygen used for respiration in some submerged vascular plants is 5% of full sunlight on clear summer days. It is estimated that 25 to 50 percent of full sunlight is necessary for many green aquatic plants to reach maximum photosynthesis. The ORSANCO committee observed that the 25-percent level of solar radiation is not reached in many of the larger streams and they considered it desirable to restrict the addition of any substances that reduce light penetration and hence limit the primary productivity of aquatic vegetation.

Recommendation: For effective photosynthetic production of oxygen, it has been found that at least 10 percent of incident light is required. Therefore, 10 percent of the incident light should reach the bottom of any desired photosynthetic zone in which adequate dissolved oxygen levels are to be maintained.

Floating materials

Floating materials include sawdust, peelings and other cannery wastes, hair and fatty materials from tanneries, wood fibers, containers, scums, oil, garbage, floating materials from untreated municipal and industrial wastes, tars and greases, and precipitated chemicals.

Wastes from paper mills, vinegar plants, cane mills, and other industries may contribute nutrients or produce conditions in streams that foster the growth of *Sphaerotilus* (Chlamydobacteriales) or similar iron or sulfur bacteria. These floating growths not only clog fishermen's nets, but also smother out the spawning grounds and habitat of all forms of aquatic life.

Recommendation: All such floating and settleable substances should be excluded from streams and lakes

Tainting substances

Among the materials that are responsible for objectionable tastes in fish are hydrocarbons, phenolic compounds, sodium pentachlorophenate (used for slime control in cooling towers), coal tar wastes, gas wastes, sewage containing phenols, coal-coking wastes, outboard motor exhaust wastes, and petroleum refinery wastes. Kraft paper mill wastes, sulfides, mercaptans, turpentine, wastes from synthetic rubber and explosives factories, algae, resins and resin acids also contribute to objectional tastes in fish. Twenty gallons per acre of kerosene or diesel fuel will produce an off-flavor in bass and bluegills which persists for 4 to 6 weeks. The Aquatic Life Advisory Committee of ORSANCO in its Third Progress Report (1960), lists the concentrations (table III-3) of phenolic substances that cause taste and odor. Albersmeyer and Erichsen (1959) found that carbolated oil and light oil, both dephenolated, impart a taste to fish flesh more pronounced than that caused by naphthalene and methyl naphthalene. They concluded that the hydrocarbons are more responsible for tastes in fish flesh than the phenolic compounds. Boetius (1954) found that chlorophenol could produce unpleasant flavor in fish at a concentration of only 0.0001 mg/l.

TABLE III–3. Concentration of Phenolic Compounds That Cause Tainting of Fish Flesh

After Bandt (1955) page 77 (except for phenol).

Compound or waste	Concentration affecting taste and odor (mg/l)	Fish tested
Pure compounds:		
Phenol	15 to 25	Trout, carp, tench, chub, eel, minnow, perch, bluegill, pike, goldfish.
Cresols	10	Tench, carp, eel, trout, minnow.
Xylenols	1 to 5	Roach, perch, carp.
Pyrocatechol	2 to 5	Perch, carp, roach.
Pyrogallol	20 to 30	Roach, carp.
P-Quinone	0.5	Carp, tench, roach.
Pyridine	5	Roach, carp.
Naphthalene	1	Roach.
Alpha Naphthol	0.5	Roach, carp.
Quinoline	0.5 to 1.0	do.
Chlorophenol	0.1	
Mixed phenolic wastes:		
Coal-coking wastes	0.02 to 0.1	Freshwater fish.
Coal-tar wastes	0.1	do.
Phenols in polluted river	0.02 to 0.15	Minnows.
Sewage containing phenols	0.1	Freshwater fish.

A preliminary laboratory study (English, McDermott, and Henderson, 1963) shows that outboard motor exhaust damages the quality of water in several ways, the most noticeable of which is causing unpleasant taste and odor in the water and off-flavoring of fish flesh. A later field study, English et al. (1963a, b) and Surber et al. (1965) determined the threshold level of tainting of fish in pond and lake waters to be about 2.6 gal/acre-foot of fuel, accumulating over a 2-month period. The gasoline used was regular grade and the lubricating oil (½ pint/gal) was a popular brand of packaged outboard motor oil.

Recommendation: Materials that impart odor or taste to fish flesh or other freshwater edible products such as crayfish, clams, prawns, etc., should not be allowed to enter receiving waters at levels that produce tainting. Where it seems probable that a discharge may result in tainting of edible aquatic products, bioassays and taste panels are suggested for determining whether tainting is likely.

Radioactive materials in fresh and marine waters

Ionizing radiation, when absorbed in living tissue in quantities substantially above that of natural background, is recognized as injurious. It is necessary, therefore, to prevent excessive levels of radiation from reaching any organism we wish to preserve, be it human, fish, or invertebrate. Beyond the obvious fact that they emit ionizing radiation, radioactive wastes are similar in many respects to other chemical wastes. Man's senses cannot detect radiation unless it is present in massive amounts. Radiation can be detected, however, by means of electronic instruments and quantities present at very low levels in the environment can be measured with remarkable accuracy. Because of the potential danger, the disposal of radioactive materials has been well planned and controlled. Injuries and loss of life from disposal of radioactive materials or from accidents involving these materials have been minimal. Four factors have contributed to this safety record: (1) scientists and legislators were aware of the dangers associated with the release of radioactive materials into the environment prior to the need for disposal; (2) research has progressed to protect man against radiation effects and levels of radiation that could be released; (3) as knowledge of nuclear energy increased, standards were developed for handling, shipping, and disposing of radioactive substances; and (4) an extensive monitoring program was inaugurated and has been functioning for years.

Upon introduction into an aquatic environment, radioactive wastes can: (1) remain in solution or in suspension, (2) precipitate and settle to the bottom, or (3) be taken up by plants and animals. Immediately upon introduction of radioactive materials into the water, certain factors interact to dilute and disperse these materials, while simultaneously other factors tend to concentrate the radioactivity. Among those factors that dilute and disperse radioactivity are currents, turbulent diffusion, isotopic dilution, and biological transport. Radioactivity is concentrated biologically by uptake directly from the water and passage through food webs, chemically and physically by adsorption, ion exchange, coprecipitation, flocculation, and sedimentation.

Radioactive wastes in the aquatic environment may be cycled through water, sediment, and the biota. Each radionuclide tends to take a characteristic route and has its own rate of movement from component to component prior to coming to rest in a temporary reservoir, one of the three components of the ecosystem. Isotopes can move from the water to the sediments or to the biota. In effect, the sediments and biota compete for the isotopes in the water. Even though in some instances sediments are initially successful in removing large quantities of radionuclides from the water, and thus preventing their immediate uptake by the biota, this sediment-associated radioactivity may later affect many benthic species by exposing them to radiation. Also, any radioactivity leached from the sediments back to the water again becomes available for uptake by the biota. Even before the radioactivity is leached from the sediment, it may become available to the biota due to a variation in the strength of the bonds between the different radionuclides and the sediment particles. Loosely bound radionuclides can be "stripped" from particles of sediment and utilized by bottom-feeding organisms.

Plants and animals, to be of any significance in the cycling of radionuclides in the aquatic environment, must accumulate the radionuclide, retain it, be eaten by another organism, and be digestible. However, even if an organism accumulates and retains a radionuclide and is not eaten before it dies, the radionuclide will enter the "biological cycle" through organisms that decompose the dead organic material into its elemental components. Plants and animals that become radioactive in this biological cycle can pose a health hazard when eaten by man.

Aquatic life may receive radiation from radionuclides present in the water and substrate and also from radionuclides that may accumulate within their tissues. Humans can acquire radionuclides via many pathways, but among the most important are drinking water or edible fish and shellfish that have concentrated nuclides from the water. In order to prevent unacceptable doses of radiation from reaching humans, fish, and other important organisms, the concentrations of radionuclides in water, both fresh and marine, must be restricted.

The effects of radiation on organisms have been the subject of intense investigation for many years. Careful consideration of pertinent portions of the vast amount of available information by such organizations as the International Commission on Radiological Protection (ICRP), the National Committee on Radiation Protection and Measurements (NCRP), and the Federal Radiation Council (FRC) has resulted in recommendations on the maximum doses of radiation that people may be allowed to receive under various circumstances (U.S. Department of Commerce, 1963). The recommended levels for the general public are substantially more conservative than those for persons who work with radiation sources or radionuclides, but in both cases the recommended levels assume that the exposure will be sustained essentially throughout the life or period of employment of the person.

The ICRP and NCRP have calculated the quantities of individual radionuclides that a person can ingest each day without accumulating levels in various body organs that deliver radiation doses in excess of the recommended limits. These quantities contained in the volume of water ingested daily (2.2 liters) are referred to as "maximum permissible concentrations (MPC) in water." The FRC, recognizing that people may ingest radionuclides from foods and other sources as well as from drinking water, has provided guidance on the basis of transient rates of intake from all sources, but only for a few nuclides (radium-226, iodine-131, strontium-90, and strontium-89).

The PHS Drinking Water Standards (USDHEW, 1962) are responsive to the recommendations of the FRC, ICRP, and NCRP, and provide appropriate protection against unacceptable radiation dose levels to people where drinking water is the only significant source of exposure above natural background. Where fish or other fresh or marine products that have accumulated radioactive materials are used as food by humans, the concentrations of the nuclides in the water must be further restricted to provide assurance that the total intake of radionuclides from all sources will not exceed the recommended levels.

The radiation dose received by fish and other aquatic forms will be greater than that received by people who drink the water or eat the fish. Even so, this does not place the fish in risk of suffering radiation damage. The radiation protection guides for people have been established with prudence, for continued exposure over a normal life span, and with appropriate risk (safety) factors. Virtually all of the available evidence shows that the concentrations of radionuclides in fish and shellfish that would limit their use as food are substantially below the concentrations that would injure the organisms from radiation. Therefore, at this time there appears to be no need for establishing separate criteria for radioactive materials in water beyond those needed to limit the intake to humans.

Recommendation: (1) No radioactive materials should be present in receiving waters as a consequence of the failure of an installation to exercise practical and economical controls to minimize releases. This recommendation is responsive to the recommendations of the FRC that: "There can be no single permissible or acceptable level of exposure without regard to the reason for permitting the exposure. It should be general practice to reduce exposure to radiation, and positive effort should be carried out to fulfill the sense of these recommendations. It is basic that exposure to radiation should result from a real determination of its necessity."

(2) No radionuclide or mixture of radionuclides should be present at concentrations greater than those specified in the PHS Drinking Water standards (USDHEW, 1962). This recommendation assures that people will receive no more than acceptable amounts

> of radioactive materials from aquatic sources and that fish living in the water will not receive an injurious dose of radiation.
>
> (3) The concentrations of radioactive materials present in fresh, estuarine, and marine waters should be less than those that would require restrictions on the use of organisms harvested from the area in order to meet the Radiation Protection Guides recommended by the Federal Radiation Council.

This recommendation assures that fish and other fresh water and marine organisms will not accumulate radionuclides to levels that would make them unacceptable for human food. It also limits the radiation dose that the organisms would receive from internally deposited nuclides to levels below those that may be injurious. Some workers (Carritt, 1959; Isaacs, 1962; Pritchard, 1959) have recommended "maximum permissible levels for sea water" based on various assumptions of dispersion, uptake by marine organisms, and the use of the organisms as food by people. While these recommendations are most useful as a first approximation in predicting safe rates of discharge of radioactive wastes, their applicability as water quality criteria is limited and they are not intended for use in fresh or estuarine waters where the concentrations of a great variety of chemical elements vary widely. Because it is not practical to generalize on the extent to which many of the important radionuclides will be concentrated by fresh water and marine forms, nor on the extent to which these organisms will be used for food by people, no attempt is made here to specify MPC for either sea water or fresh water in reference to uptake by the organisms. Rather, each case requires a separate evaluation that takes into account the peculiar features of the region. Such an evaluation should be approved by an agency of the State or Federal Government in each instance of radioactive contamination in the environment. In each particular instance of contamination, the organisms present, the extent to which these organisms concentrate the radionuclides, and the extent to which man uses the organisms as food must be determined, as well as the rates of release of radionuclides must be based on this information.

Plant nutrients and nuisance organisms

All terrestrial biological processes plus the majority of man's activities ultimately result in waste products in various stages of decomposition. A portion of these sooner or later enter surface freshwaters. These waste products include a rather abundant amount of plant nutrients such as nitrogen, phosphorus, carbon, and other elements. Subsequently, these plant nutrients are incorporated into organic matter by aquatic plants.

Surface water areas are like land areas in that some type of vegetation will occupy any suitable habitat. Thus, the more abundant the nutrient supply, the more dense the vegetation, provided other environmental factors are favorable. In the aquatic habitat, these growths may be bacteria, aquatic fungi, phytoplankton, filamentous algae, submersed, emersed, floating, and marginal water plants. Practically all aquatic plants may be desirable at one time or another and in one habitat or another. However, when they become too dense or interfere with other uses of the water or of the aquatic habitat, they become nuisance growths.

Some sheath-forming bacteria are the primary nuisance-type growths in rivers, lakes, and ponds. A notable problem associated with this group occurs in areas subjected to organic enrichment. The most common offenders belong to the genus *Sphaerotilus*. These bacteria are prevalent in areas receiving raw domestic sewage, improperly stabilized paper pulp effluents or effluents containing simple sugars. The growths they produce interfere with fishing by fouling lines, clogging nets, and generally creating unsightly conditions in the infested area. Their metabolic demands while they are living and their decomposition after death impose a high BOD load on the stream and can severely deplete the dissolved oxygen. It has been suggested that large populations of *Sphaerotilus* render the habitat noxious to animals and hence its presence may actively exclude desirable fish and invertebrates.

The freshwater algae are diverse in shape, color, size, and habitat. A description of all species of algae would be as comprehensive as writing about all land plants, mosses, ferns, fungi, and seed plants.

They may be free floating (planktonic) or they may grow attached to the substrate (benthic or epiphytic types). They may be macroscopic or microscopic and are single-celled, colonial, or filamentous. They are the basic link in the conversion of inorganic constituents in water into organic matter. When present in sufficient numbers, these plants impart a green, yellow, red, or black color to the water. They may also congregate at or near the water surface and form so-called "waterbloom" or "scum."

A major beneficial role of algae is the removal of carbon dioxide from the water by photosynthesis during daylight and the production of oxygen. Algae, like other organisms, continually respire and produce carbon dioxide. The amount of oxygen produced during active photosynthesis is many times the amount of carbon dioxide released during the night or on cloudy days when photosynthesis is inhibited or stopped.

Limited concentrations of algae are not troublesome in surface waters; however, overproduction of various species is considered undesirable for many water uses. A relatively abundant growth of planktonic algae in waters 3 feet or deeper will shade the bottom muds sufficiently to prevent germination of seeds and halt the growth of practically all rooted submersed and emersed aquatics, thus removing an important source of food for ducks and other water fowl.

Some blue-green algae, many green algae, and some diatoms produce odors and scums that make waters less desirable for swimming. Dense growths of such planktonic algae may limit photosynthetic activity to a layer only a few inches beneath the surface of the water. Under certain conditions, the populations of algae may die and their decomposition will deplete dissolved oxygen in the entire body of water. Certain sensitive people are allergic to many species of planktonic algae blooming in waters used for swimming.

It is claimed that some species of algae cause gastric disturbances in humans who consume such infested waters. Several species of blue-green algae produce, under certain conditions, toxic organic substances that kill fish, birds, and domestic animals. Some of the genera that contain species which may produce toxins are *Anabaena*, *Anacystis*, *Aphanizomenon*, *Coleolosphaerium*, *Gloeotrichia*, *Microcystis*, *Nodularia*, and *Nostoc*. Some species of *Chlorella*, a green alga, also are toxic.

Various species of single, as well as branched filamentous forms of algae, grow in both cool and warm weather and when they become overabundant are generally considered to be a nuisance in whatever body of water they occur. Most species of these algae are generally distributed over the United States.

Many forms of plankton and filamentous algae clog sand filters in water treatment plants, produce undesirable tastes and odors in drinking water, and secrete oily substances that interfere with domestic use and manufacturing processes. Some algae cause water to foam during heating as well as metal corrosion and the clogging of screens, filters, and piping. Algae also coat cooling towers and condensers causing these units to become ineffective. In Lake Superior, complaints have been made that diatoms such as *Tabellaria*, *Synedra*, *Cymbella*, and *Fragilaria*, and the chrysophyte, *Dinobryon*, may be the cause of slimes on fishnets.

Filamentous algae may interfere with the operation of irrigation systems by clogging ditches, wires, and screens and thus seriously impede the flow of water. Filamentous algae in ponds, lakes, and reservoirs may cause depletion of naturally occurring and added nutrients that could otherwise be used to produce unicellular algae that are more commonly used as food by fish. Dense growths of filamentous algae may reduce the total fish production and seriously interfere with harvesting the fish either by hook and line fishing, seining, or draining. Such growths can also cause overpopulation, resulting in stunting and the presence of large numbers of small fish. Under certain conditions, growths of filamentous algae on pond or lake bottoms become so dense that they eliminate spawning areas of fish and possibly interfere with the production of invertebrate fish food.

Submersed plants are those which produce all or most of their vegetative growth beneath the water surface. In many instances these plants have an underwater leaf form, a totally different floating or emersed leaf form, and flowers on an aerial stalk. Abundant growth of these weeds is dependent upon depth and turbidity of water, and substratum. For most submersed plants in clear water, 8 to 10 feet is the maximum depth for growth in clear water as they must receive sufficient light for photosynthesis when they are seedlings. Most of these submersed aquatic plants appear capable of absorbing nutrients as well as herbicides through either their roots or vegetative parts.

Emersed plants are rooted in bottom muds and produce a majority of their leaves and flowers at or above the water surface. Some species have leaves that are flat and float entirely upon the water surface. Other species have leaves that are saucer-shaped or whose margins are irregular or fluted. The latter types of leaves do not float entirely upon the water surface.

Marginal plants are probably the most widely distributed of the rooted aquatic plants. Members of this group are varied in size, shape, and preference of habitat. Many species are adapted for growth from moist soils into water up to 2 feet deep or more. Other species are limited to moist soil or entirely to a watery habitat.

There are some species of floating plants that are rather limited in their distribution while others are widespread throughout the world. Plants in this group have true roots and leaves, but instead of being anchored in the soil they float about on the water surface. Buoyancy of the plant is accomplished through modification of the leaf (including covering of the leaf surface) and leaf petiole. Most species have well-developed root systems which collect nutrients from the water.

Species designated as weeds are not necessarily such in all places and at all times. For example, many submersed, floating, and emersed plants that normally interfere with boating, swimming, and fishing are regarded as desirable growths in waterfowl refuge areas. Rooted plants with floating leaves, such as water lillies and watershield, and those that float upon the surface, such as water hyacinth, elodea, parrotweed, alligatorweed, and duckweed, are considered highly objectionable for many water uses. In clear water areas, however, where artificial or natural fertilization is moderate, the removal of these surface-shading plants may permit sunlight to penetrate to the bottom muds and submersed plants soon will occupy these waters. These submersed plants generally are more objectionable in an area than the original surface-covering plants.

Most emersed, marginal, and a few submersed plants and filamentous algae produce growths that provide a suitable habitat for the development of anopheline and other pest-type mosquitoes as well as a hiding place for snakes. They are excellent habitats for damselflies and some aquatic beetles.

Most rooted and floating aquatic plants can seriously interfere with navigation of small recreational craft and large commercial boats in infested areas. Such problems are prevalent in intercoastal waterways and in some streams in the Gulf States area. Water shortages due to consumption by undesirable aquatic plants or reduction in carrying capacity of an irrigation or drainage canal through excessive vegetation can result in decreased crop quality, yield, or even crop failure.

Submersed and emersed weeds consume nutrients, either available or added, that could otherwise be used to grow desirable planktonic algae in impounded waters. Thus, the presence of excessive rooted plants may reduce total fish production in the infested body of water. Extensive growth of weeds provides dense cover that allows the survival of excessive numbers of fish resulting in overcrowding and stunting as well as interfering with harvesting the fish by hook and line or other methods. There is evidence that rank growths of submersed, emersed, or floating weeds may deplete the dissolved oxygen supply in shallower water and that fish tend to leave these areas if there are open-water areas available of better quality. Although they carry on the process of photosynthesis, their multicellular structure often makes them less effective in re-oxygenating the water.

All the elements essential for plant growth are yet to be determined. Some of the elements known to be important are nitrogen, phosphorus, potassium, magnesium, calcium, manganese, iron, silicon (for diatoms), sulfur (as sulfates), oxygen, and carbon. In many habitats, abundance of the first two elements, N (nitrogen) and P (phosphorus), promotes vegetative production if other conditions for growth are favorable. Most algae also require some simple organics, such as amino acids and vitamins, and many trace elements, such as manganese and copper. Not only are the various factors important, but their relative abundance and combined affect can be of even greater importance. Limited laboratory studies made to date indicate that different species of algae have somewhat different phosphorus requirements with the range of available phosphorus usually falling between 0.01 and 0.05 mg/l as phosphorus. At these levels, when other conditions are favorable, blooms may be expected. As has been pointed out by the Subcommittee on Water Quality Criteria for Public Water Supply, the total phosphorus is of outstanding importance. While there is no set relationship between total and available phosphorus (because the ratio varies with season, temperature, and plant growth), the total phosphorus is governing as it is the reservoir that supplies the available phosphorus. It is believed that allowable total phosphorus depends upon a variety of factors; e.g., type of water, character of bottom soil, turbidity, temperature, and especially desired water use. Allowable amounts of total phosphorus will vary, but in general it is believed that a desirable guideline is 100 µg/l for rivers and 50 µg/l where streams enter lakes or reservoirs (recommended by the Public Water Supply Subcommittee).

The nitrogen-phosphorus ratio is also of importance. The ratio varies with the water, season, temperature, and geological formation, and may range from 1 or 2:1 to 100:1. In natural waters, the ratio is often very near 10:1, and this appears to be a good guideline for indicating normal conditions.

The major sources of nitrogen entering fresh waters are atmospheric (approximately 5 lbs/acre/year), (Hutchinsen, 1957), domestic sewage effluents, animal and plant processing wastes, animal manure, fertilizer and chemical manufacturing spillage, various types of industrial effluents, and agricultural runoff.

The major sources of phosphorous entering fresh waters are domestic sewage effluents (including detergents), animal and plant processing wastes, fertilizer and chemical manufacturing spillage, various industrial effluents, and, to a limited extent, erosion materials in agricultural runoff.

Phosphorous entering an ecosystem may produce a high oxygen demand. It has been pointed out that 1 milligram of phosphorous from an organic source demands about 160 milligrams of oxygen in a single pass through the phosphorus cycle to complete oxidation. Thus the oxidation of organic matter, the growth of which has been induced by adding phosphorus, may bring about a great reduction of oxygen in a lake or stream.

Dissolved carbon in the form of simple organic compounds can be utilized by many kinds of algae. These types of carbon compounds are also used directly as a source of food by many animals. Varying amounts of simple organic compounds containing carbon are found in sewage and several types of industrial wastes. Other more complex forms of organic carbon can be utilized by bacteria. The most common nuisance growth that becomes very abundant in the presence of very small amounts of carbon is *Sphaerotilus*. Patrick (unpublished data) has shown that the addition of 0.05–0.1 mg/l of glucose, without changing other ecological conditions, may produce nuisance growths.

Knowledge of the nutrient requirements of fungi, phytoplankton, and filamentous algae is more extensive than for rooted aquatic plants. Laboratory data on nutrient requirements must be used with caution, however, because the maintenance of most long-term cultures has required that extracts of soil be incorporated into the inorganic culture medium. Analyses of field grown algae have indicated a wide divergence in elemental composition among various species and among the same species from different localities. Excessive growths often seen to be triggered by small amounts of so-called minor or trace elements and vitamins, particularly B_{12}.

One of the most obvious effects of increases or imbalances in nutrients is the change in the kinds and abundance of species composing the algal flora. Historical studies of Lake Erie show a change from an *Asterionella* dominance in the spring and a *Synedra* dominance in the fall of 1920 to a *Melosira* dominance in the spring and a *Melosira, Anabaena, Oscillatoria* dominance in the fall of 1962. Between 1919 and 1934, the number of cells per ml, with two exceptions, always were less than 4,000/ml. Since 1934, the cell count, with one exception, has always been greater than 4,000/ml. In 1944, it reached 11,032 cells/ml. It should be pointed out that blue-green algae are a poor source of food for most aquatic life.

Benthic forms also indicate the increase in nutrients in an ecosystem. Various species of *Cladophora* become abundant in lakes and rivers when nutrients are abundant and replace the original diverse benthic flora.

This demand for a wide variety of nutrients is also characteristic of many of the rooted aquatic plants. Their affinity for numerous metals, however, does not appear to be comparable to that of the algae.

Extensive data exist on the concentration of nitrogen and phosphorus in fresh waters throughout the United States. (Allee, et al., 1949; Ellis, 1940; Engelbrecht and Morgan, 1961; Juday, et al., 1927; Lackey, 1945; USDHEW, 1962a) In evaluating these data, it must be remembered that algae and most other aquatic plants are capable of utilizing any available N and/or P in a very short time providing other growth conditions are favorable. Thus, analyses of filtered water would not provide an evaluation of all elements existing in the original water sample. A more meaningful figure would result if all materials in an original water sample were digested and then analyzed. Often, the dissolved or available phosphorus may be very low, while the total amount in the organisms and organic matter may be quite large. Not only does this determination of total phosphorous give a better estimate of the existing nutrient load of an area, but it also provides an index to the potential release that would occur if these plants should all die within a short period of time.

This information would also point out the fact that in many freshwaters, various species of rooted aquatic plants are excellent receptors for this nutrient load. Their use in effluent treatment might be one of the cheaper waste-treatment procedures. The chemical composition of several species of plants is given in table III–4. Indications are that the N–P content of freshwaters in the United States is quite varied, and their presence in fairly large amounts may or may not produce algae blooms.

It must be remembered that factors other than plant nutrients also are operative in the establishment and maintenance of aquatic plant growths. There must be sufficient light reaching the plant for photosynthesis to occur. If turbidity from muds, dyes, other materials, or even phytoplankton is too great, plants at lower depths cannot grow. These same plants, however, if established in an area, can trap large amounts of intermittent silt and other materials and clear the waters for downstream uses.

Another factor that might be operative in preventing aquatic plant growth would be the lack of free CO_2 and bicarbonate ions in a particular aquatic environment. Certainly in an area where the pH is high (9.5 or above) or low (below 5.5), productivity would not reach high levels due to a lack of sufficient bicarbonates.

Temperature also is an important factor in determining the amount of growth. For each species, there is an optimum range in which the greatest growth occurs.

Wave action on large expanses of water may also be a factor in regulating all types of aquatic plant growths. This appears contradictory to the concept that winds cause mixing of surface and bottom waters, thereby renewing plant nutrients in the euphotic zone. However, in certain lakes and reservoirs, wind-induced waves and currents mechanically agitate bottom materials and waters to an extent that interferes with the production of phytoplankton and rooted aquatic plants.

Various workers have discussed the concentrations of nitrogen and phosphorus that are needed for an algal bloom. Sawyer (1947) suggests that a concentration of at least 15 μg/l of phosphorus is necessary for growth. Hutchinson (1957) states that *Asterionella* can take up phosphorus from where it is present at less than one μg/l. As a result of the study of 17 Wisconsin lakes, Mackenthun (1965) cites results indicating that inorganic nitrogen at 0.30 mg/l and inorganic phosphorus at 0.01 mg/l, at the start of an active growing season, subsequently permitted algal blooms. As yet, there is no definite information on the amount of wastes that will produce predictable harmful effects in a lake. There are indicators, however, of developing or potentially undesirable conditions.

There are several conditions, analyses, or measures that will indicate eutrophication and dystrophication. Since these parameters are not infallible, it is well to use them in combinations. Conditions indicative of organic enrichment are:

(1) A slow overall decrease year after year in the dissolved oxygen in the hypolimnion as indicated by determinations made a short time before the fall overturn and an increase in anaerobic areas in the lower portion of the hypolimnion.

TABLE III–4. Chemical Composition of Some Algae From Ponds and Lakes in Southeastern United States [1]

Analysis	Chara	Pithophora	Spirogyra	Giant Spirogyra	Rhizoclonium	Oedogonium	Mougeotia	Anabaena
Ash percent	43.4	27.77	13.06	13.86	17.36	12.69	14.54	5.19
C percent	29.3	35.38	42.40	41.16	39.10	40.84	40.74	49.70
N percent	2.46	2.57	3.01	2.35	3.46	2.64	1.77	9.43
P percent	0.25	0.30	0.20	0.23	0.43	0.08	0.25	0.77
S percent	0.55	1.42	0.27	0.24	0.27	0.15	0.36	0.53
Ca percent	8.03	3.82	0.57	0.84	0.52	0.44	1.68	0.36
Mg percent	0.92	0.20	0.45	0.30	0.21	0.16	0.57	0.42
K percent	2.35	3.06	0.92	0.99	1.90	3.03	1.20	1.20
Na percent	0.13	0.07	1.42	1.43	0.09	0.06	0.49	0.18
Fe mg/l	2,520	2,836	1,368	1,793	1,820	1,645	60	80
Mn mg/l	2,926	829	1,641	1,658	1,687	1,729	1,080	800
Zn mg/l	89	29	72	46	89	119	520	---
Cu mg/l	19	23	47	34	75	75	143	70
B mg/l	6.7	65	4.2	4.3	1.8	8.1	8	---

Analysis	Cladophora	Euglena	Hydrodictyon	Microcystis	Lyngbya	Nitella	Aphanizomenon
Ash percent	23.38	4.12	17.94	6.2	17.20	19.11	7.21
C percent	35.27	48.14	39.96	46.46	40.23	38.43	47.65
N percent	2.30	5.14	3.87	8.08	5.01	2.70	8.57
P percent	0.56	0.67	0.24	0.68	0.31	0.23	1.17
S percent	1.58	0.19	1.41	0.27	0.28	0.34	1.18
Ca percent	1.69	0.05	0.69	0.53	0.45	1.89	0.73
Mg percent	0.23	0.07	0.17	0.17	0.14	0.95	0.21
K percent	6.08	0.34	4.21	0.79	0.42	3.73	0.66
Na percent	0.18	0.02	0.38	0.04	0.06	0.28	0.19
Mn mg/l	1,040	240	1,373	2,751	5,230	2,388	167
Fe mg/l	2,300	1,545	1,963	322	3,866	2,180	833
Zn mg/l	10	73	129	48	171	240	120
Cu mg/l	190	290	114	37	101	39	187
B mg/l	84.6	3.8	---	3.6	112	9.8	---

[1] Lawrence (personal communication).

(2) An increase in dissolved solids—especially nutrient materials such as nitrogen, phosphorus, and simple carbohydrates.

(3) An increase in suspended solids—especially organic materials.

(4) A shift from a diatom-dominated plankton population to one dominated by blue-green and/or green algae, associated with increases in amounts and changes in relative abundance of nutrients.

(5) A steady though slow decrease in light penetration.

(6) An increase in organic materials and nutrients, especially phosphorus, in bottom deposits.

Recommendation: The Subcommittee wishes to stress that the concentrations set forth are suggested solely as guidelines and the maintenance of these may or may not prevent undesirable blooms. All the factors causing nuisance plant growths and the level of each which should not be exceeded are not known.

(1) In order to limit nuisance growths, the addition of all organic wastes such as sewage, food processing, cannery, and industrial wastes containing nutrients, vitamins, trace elements, and growth stimulants should be carefully controlled. Furthermore, it should be pointed out that the addition of sulfates or manganese oxide to a lake should be limited if iron is present in the hypolimnion as they may increase the quantity of available phosphorus.

(2) Nothing should be added that causes an increased zone of anaerobic decomposition of a lake or reservoir.

(3) The naturally occurring ratios and amounts of nitrogen (particularly NO_3 and NH_4) to total phosphorus should not be radically changed by the addition of materials. As a guideline, the concentration of total phosphorus should not be increased to levels exceeding 100 μg/l in flowing streams or 50 μg/l where streams enter lakes or reservoirs.

(4) Because of our present limited knowledge of conditions promoting nuisance growth, we must have a biological monitoring program to determine the effectiveness of the control measure put into operation. A monitoring program can detect in their early stages the development of undesirable changes in amounts and kinds of rooted aquatics and the condition of algal growths. With periodic monitoring such undesirable trends can be detected and corrected by more stringent regulation of added organics.

Toxic substances

Aquatic life too frequently is considered only in terms of harvestable species. The fact that numerous other organisms are essential to produce a crop of fishes often is overlooked or given little attention. To produce a harvestable crop of fish, it is essential to have supporting plants and animals for food. Requirements are established on the basis that the needed criteria are those that will protect fish, the harvested crop, and the food organisms necessary to support that crop. At this time, it is believed that every important species should be protected. One can appreciate that unimportant organisms may be sacrificed if the following criteria are adopted. Fish too often are considered as a single species instead of a multitude of species. Many are distinctly and greatly different from other related species and have their own distinctive requirements. Because of this, and because the important species, *essential* food organisms, and water quality will be different in different habitats, a single value or concentration has very limited applicability unless appropriate margins of safety are incorporated.

For these reasons, the bioassay approach described later in this section is favored. It is believed that bioassays are the best method for determining safe concentrations of toxicants for the species of local importance. Bioassays are essential also to determine safe concentrations for food organisms of those species and the effect of existing water quality, including environmental variables as well as existing pollution. Pertinent to this stance is the fact that the majority of specific pollution problems are ones involving discharges of unknown and variable composition. Almost without exception, more than one toxicant or stress is present. Further, suggested safe concentrations probably will not be adequate in instances where more than one adverse factor exists. It is believed that these recommended levels will be adequate for a particular pollutant if dissolved oxygen, temperature, and pH are within the limits recommended. If the latter parameters are outside recommended limits, appropriate alterations in the criteria for toxicants must be made.

Most of the available toxicity data are reported as the median tolerance limit (TL_m), the concentration that kills 50 percent of the test organisms within a specified time span, usually in 96 hours or less. This system of reporting has been misapplied by some who have erroneously inferred that a TL_m value is a safe value, whereas it is merely the level at which half the test organisms are killed. In many cases, the differences are great between TL_m concentrations and concentrations that are low enough to permit reproduction and growth.

Substantial data on long-term effects and safe levels are available for only a few toxicants, perhaps 10. The effect of toxicants on reproduction is nearly unknown, yet this is a very important aspect of all long-term toxicity tests. In chronic tests with six different toxicants, there were three toxicants with which certain concentrations permitted indefinite survival and normal appearance but blocked spawning completely. Such evidence makes estimates of safe concentrations based on acute lethal test data alone very difficult and frequently erroneous. Equally problematical is the near-total lack of information on the sensitivity of the various life stages of organisms. Many organisms are the most sensitive in the larval, nymph, molting, or fry state; some may be the most sensitive in the egg and sperm stage.

A further difficulty is encountered in recommending criteria because continuous acceptable concentrations must be lower than the intermittent concentrations that may be reached occasionally without causing damage. There seems to be only one way in which to resolve this difficulty and that is to use *both* maximum concentration and a range of concentrations. It is recognized that the extremes do limit organisms, but, within these extremes there is a range in concentration that can be tolerated and is safe for prolonged periods of exposure.

Average 24-hour concentrations can be determined by using a small water pump to collect 1 to 5 ml samples every minute. After 24 hours, the sample is mixed and analyzed. The concentration found represents the average concentration. Samples obtained this way are more reproducible and easier to secure than the maximum instantaneous concentration. Maximum concentrations must be considered in the criteria, however, because an average concentration alone could be met and yet permit a lethal concentration to exist for a critical period.

Bioassay

The use of some type of bioassay to determine the toxicity of a material or waste can be the most effective and accurate method of assessing potential danger. With these methods, no assumptions need be made concerning the chemical structure or form of the pollutant, nor does the investigator have to know the constituent substances. The effects of water quality on toxicity also may be measured. Naturally, the more that is known about the chemical and physical behavior of a toxicant in water, the more precise the assay can be.

While there are many types of assays, two are in general use: (1) the static bioassay in which the test solution is not changed during the period of exposure, and (2) the flow-through bioassay in which the test solution continually is renewed. It is nearly impossible in a static test to use the introduced test concentration for calculating TL$_m$ values, especially for substances or wastes that are toxic at concentrations of 1 mg/l or less, because the quantity taken into the test organism may be a very large percentage of the amount contained in the test water. A 48-hour TL$_m$ based on the introduced concentration could give a value much higher than the true concentration because of this decrease in toxicant concentration. The initial test concentration is usually not measured in static tests because of the changing concentration. Knowledge of the concentration of the toxicant at the end of the test can be of value.

The static test can give useful relative measures of toxicity for wastes of high toxicity, but for the reason mentioned above, it should not be used for absolute values. Less toxic substances can be assayed much more accurately and lethal concentrations can be determined with confidence. The chemical nature of the tested substances has an important effect on the accuracy of the results as well. Substances that are volatile, unstable, or relatively insoluble may not be accurately assayed while substances having opposite properties can be assayed more accurately.

The problem of maintaining oxygen concentrations suitable for aquatic life in the test chamber can be very difficult. Insufficient oxygen may be present in the test water volume because a BOD or COD may consume much of the available dissolved oxygen and aeration or oxygenation may degrade or remove the test material. Devices for maintaining satisfactory dissolved oxygen in static tests have been proposed and used with some degree of effectiveness. A rather complete account of static assays can be found in Sandard Methods for the Examination of Water and Wastewater, 12th edition (1965), and Doudoroff, et al. (1951).

In the flow-through type of bioassay a device is used to add toxicant to a flow of water and the mixture is discharged into the test container. This method of testing has few of the problems mentioned in connection with the static test and has other advantages in addition. Its important disadvantage is the more complicated work of building the necessary equipment; namely, a water supply system, metering devices, and the provision of a large quantity of the test substance.

Its important advantages are that a predetermined concentration of test material can be maintained, oxygen concentrations can be kept high or be controlled, metabolites and waste products are removed (animals can be fed), absolute rather than relative TL$_m$ values can be obtained, and volatile, unstable, and sparingly soluble materials can be tested. Additionally, multifactor experiments are possible in which several variables can be controlled (pH, dissolved oxygen, carbon dioxide, etc.). The constant renewal test is superior for monitoring effluents, water supplies, or streams on a continuous or intermittent basis and is the only suitable method for long-term tests.

Several systems for adding the test material to the water have been devised since this type of bioassay has been in use. Lemke and Mount (1963) describe a system using a controlled water flow balanced against a chemical metering pump. Henderson and Pickering (1963) describe a simple drip system and a controlled water flow; a similar system is proposed by Jackson and Brungs (1966). Both of these latter references describe the use of fish and flowing systems as continuous monitors. Mount and Warner (1965), Mount and Brungs (1967), and Brungs and Mount (1967) describe systems suitable for continuous short or long-term tests.

Most criteria for toxic substances must be based on a bioassay made for each specific situation. This is dictated by the lack of information and the wide variation in situations, species, water quality, and the nature of the substance being added to the water.

Most of the bioassay work on algae has measured the threshold concentrations that reduce physiological processes by 50 percent rather than the concentrations that cause 50-percent death in the population tested. It is very difficult to determine the death point of algae cells, but some workers have used it as a criterion. Physiological measurements have been based largely on 50-percent reduction in photosynthesis and 50-percent reduction in number of divisions that have taken place during a period of time. This is determined by the number of cells present at the beginning and end of the experiment. A bioassay method employing diatoms has been recognized by the American Society for Testing Materials (1964).

Application Factors

Short-term or acute toxicity tests provide information on the overall toxicity of a material and thus precede meaningful long-term toxicity studies. They may also be used to compare toxicities of different materials. When water for dilution is taken from the receiving stream, these tests may also indicate additional stresses due to materials already present in the receiving water. These acute studies do not indicate concentrations of a potential toxicant that are harmless under conditions of long-term exposure. It is desirable, therefore, to develop a factor that can be used with 96 or 48-hour TL_m values to indicate concentrations of the waste or material in question that are safe in the receiving water. Such a factor has been called an application factor.

Ideally, an application factor should be determined for each waste or material. To do this, it would be necessary to determine the concentration of the waste or material in question that does not adversely affect the productivity of the aquatic biota on continuous exposure, in water of known quality, and under environmental conditions (DO, temperature, pH, etc.) at which it is most toxic. This concentration is then divided by the 96-hour TL_m value obtained under the same conditions to give the application factor.

$$\frac{\text{safe concentration for continuous exposure}}{\text{96-hour } TL_m}$$

For example, if the 96-hour TL_m is 0.5 mg/l and the concentration of the waste found to be safe is 0.01 mg/l, the application factor would be:

$$\frac{\text{safe concentration}}{\text{96-hour } TL_m} = \frac{0.01}{0.50} = \frac{1}{50}$$

In this instance, the application factor is $^1/_{50}$ or 0.02. Then in a given situation involving this waste, the safe concentration in the receiving stream would be found by multiplying the 96-hour TL_m by 0.02.

To effectively determine the application factor for a given waste, it is necessary to determine the concentration of that waste which is safe under a given set of conditions. For those materials whose toxicity is not significantly influenced by water quality and in streams free of other wastes that influence the waste in question or that have water qualities similar to those under which the waste was tested, the above-mentioned concentration would be the one that is safe in the receiving water. However, differences in water quality and lack of information on the toxicity of waste materials already present make the direct use of laboratory-determined safe levels unwise at present, and a different approach is recommended.

In this approach, a 96-hour TL_m is determined for the waste using water from the receiving stream for dilution and, as test organisms, the most sensitive species or life stage of an economically important local species or one whose relative sensitivity is known. This procedure would take into consideration the effects of local quality and the stress or adverse effects of wastes already present in the stream. The TL_m value thus found then is multiplied by the application factor for that waste to determine the safe concentration of that waste in that stream or stream section. Such bioassays should be repeated at least monthly and at each change in process or rate of waste discharge.

This procedure must be used because of the extreme difference in sensitivity among species and among necessary fish food organisms. Henderson (1957) has discussed various factors involved in developing application factors. Results of studies by Mount and Stephan (1967), in which continuous exposure was used, reveal that the application factor necessary to reduce the concentration low enough to permit spawning ranged from 1/4 to 1/200. It is recognized that exposure will not be constant in most cases and that higher concentrations usually can be tolerated for short periods.

At present, safe levels have been determined for only a few wastes and hence only a few application factors are known. Since the determination of safe levels is an involved process, it will be necessary to use indirect or stopgap procedures for estimating tolerable concentrations of various wastes in receiving waters. To meet this situation, it is proposed to use three universal application factors selected on the basis of present knowledge, experience, and judgment. It is proposed that these general application factors be applied to TL_m values determined by those discharging wastes in the manner described above to set tolerable concentrations of their wastes in the receiving stream.

It should be evident that when these general application factors are used for all wastes the resulting concentrations at times will be more stringent than needed for some wastes and inadequate for others. The derived concentrations will be tolerable, however, for a considerable number of wastes in the midrange of relative toxicity.

Recommendation for the Use of Bioassays and Application Factors To Denote Safe Concentrations of Wastes in Receiving Streams: (1) For the determination of acute toxicities, flow-through bioassays are the first choice. Methods for carrying out these flow-through tests have been described by Surber and Thatcher (1963), Lemke and Mount (1963), Henderson and Pickering (1963), Jackson and Brungs (1966), Mount and Warner (1965), Mount and Brungs (1967), and Brungs and Mount (1967). Flow-through bioassays should be used for unstable, volatile, or highly toxic wastes and those having an oxygen demand. They also must be used when several variables such as pH, DO, CO_2 and other factors must be controlled.

(2) When flow-through tests are not feasible, tests of a different type or duration must be used. The kinds of local conditions affecting the procedure might be a single application of pesticides or lack of materials and equipment.

(3) Acute static bioassays with fish for the determination of TL_m values should be carried out in accordance with Standard Methods for the Examination of Water and Wastewater, 12th edition (1965). Such tests should be used for the determination of TL_m values only for persistent, nonvolatile, or highly soluble materials of low toxicity which do not have an oxygen demand because it is necessary to consider the amount added as the concentration to which the test organisms are exposed.

(4) When application factors are used with TL_m values to determine safe concentrations of a waste in a receiving water, the bioassay studies to determine TL_m values should be made with the most sensitive local species and life stages of economical or ecological importance and with dilution water taken from the receiving stream above the waste outfall. Other species whose relative sensitivity is known can be used in the absence of knowledge concerning the most sensitive of the important local species or life stages or due to difficulty in providing them in sufficient numbers. Alternatively, tests may be carried out using one species of diatom, one species of an invertebrate, and two species of fish, one of which should be a pan or game fish. Further, these bioassays must be performed with environmental conditions at levels at which the waste is most toxic. Tests should be repeated with one of the species at least monthly and when there are changes in the character or volume of the waste.

(5) Concentration of materials that are nonpersistent (that is, have a half life of less than 96 hours) or have noncumulative effects after mixing with the receiving waters should not exceed 1/10 of the 96-hour TL_m value at any time or place. The 24-hour average of the concentration of these materials should not exceed 1/20 of the TL_m value after mixing. For other toxicants the concentrations should not exceed 1/20 and 1/100 of the TL_m value under the conditions described above. Where specific application factors have been determined, they will be used in all instances.

When two or more toxic materials whose effects are additive are present at the same time in the receiving water, some reduction in the permissible concentrations as derived from bioassays on individual substances or wastes is necessary. The amount of reduction required is a function of both the number of toxic materials present and their concentrations in respect to the derived permissible concentration. An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible concentration for the mixture is through the use of following relationship:

$$\left(\frac{C_a}{L_a}+\frac{C_b}{L_b}\cdots+\frac{C_n}{L_n}\leq 1\right)$$

Where C_a, C_b, . . . C_n are the measured concentrations of the several toxic materials in the water and L_a, L_b, . . . L_n are the respective permissible concentration limits derived for the materials on an individual basis. Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary.

Heavy Metals

An extensive discussion of the physiological mode of action of heavy metals is found in the toxicity portion of the section on water quality requirements for marine organisms.

Zinc: While much information has been published regarding zinc, a large amount of the data cannot be used because of incomplete description of methods, type of water, or concentrations. The authors of many of the papers dealing with zinc toxicity have used various specific sublethal effects as endpoints and there is no way to compare these findings with other work.

Since the concentration of calcium and magnesium influences heavy metal toxicity, permissible levels of heavy metals are dependent on the calcium and magnesium concentrations. Certain studies with zinc (Mount, 1966; and unpublished work of the FWPCA National Water Quality Lab., Duluth, Minn.) and cadmium indicate that for a given calcium and magnesium concentration the acute toxicity of zinc and cadmium increases (TL_m concentration decreases) as pH is raised from 5 to 9. This seems contrary to prevalent opinion that metal toxicity is related to metal in solution and that as pH increases (solubility decreases) the toxicity decreases. The reason for this apparent contradiction is that conceptions concerning the effect of pH are based on natural waters in which pH does not vary independently of calcium and magnesium concentrations, but rather is closely related to it. In those cases where this relationship has been studied, except for one (Sprague, 1964b), the toxicity has increased with an increase in pH. This concept also is consistent with the work of Lloyd (1961b) and, more recently, that of Herbert and Wakeford (1964) who concluded that colloidal or flocculated, but suspended, zinc exerts a toxic influence on fish.

The significance of temperature and the calcium-magnesium content on the toxicity of zinc to plankton has been pointed out by Patrick (unpublished data). In these tests, a 50-percent reduction in growth of the population was used as a measure. Results of these tests are summarized as follows:

Temperature of test solution	*Concentration in mg/l which reduces growth of population by 50 percent*	
	Ca-Mg concentration—44 mg/l as $CaCO_3$ Nitzschia linearis	*Ca-Mg concentration—170 mg/l as $CaCO_3$* Navicula seminulum
72 F	4.29 mg/l	4.05 mg/l.
82 F	1.59 mg/l	2.31 mg/l.
86 F	1.32 mg/l	3.22 mg/l.

Palmer (1957) found that zinc dimethyl dithiocarbamate (ZDD) inhibited growth of *Microcystis* at 0.004 mg/l. A concentration of 0.25 mg/l controlled all diatoms, 43 percent of the blue-green algae, and 18 percent of the green algae. The above evidence implies that permissible levels of zinc cannot be related to the calcium-magnesium concentrations or to pH alone.

Herbert and Wakeford (1964) described the effect of salinity on the toxicity of zinc to rainbow trout. Since zinc was most toxic to trout in freshwater, it is assumed that concentrations which are safe in freshwater will be safe for the salmonids in brackish water. The maximum reported affect of a reduction of dissolved oxygen from 6–7 mg/l to 2 mg/l on the acute toxicity of zinc is a 50-percent increase in its acute toxicity (Lloyd, 1961a; Pickering, in press; Cairns and Scheier, 1958a). Since 4 mg/l is the minimum permitted, this effect is small in comparison to the difference between safe and acutely toxic concentrations. The use of an application factor, therefore, should provide adequate protection. Similarly, Herbert and Shurban (1963a) found that the 24-hour TL_m for zinc was reduced only 20 percent for rainbow trout forced to swim at 85 percent of their maximum sustained swimming speed.

The effect of calcium and magnesium concentrations on the toxicity of zinc for plankton, invertebrates, fishes, and their embryonic stages is reflected in the spread of values reported as toxic by many sources (Anderson 1950; Brungs, in press; Cairns and Scheier 1957, 1958b; Grande, 1966; Herbert and Shurben, 1963a, b; Jones, 1938; Lloyd, 1961b; Patrick, personal communication; Pickering, in press; Pickering and Henderson, 1966a; Pickering and Vigor, 1965; Skidmore, 1964; Sprague, 1964a, b; Sprague and Ramsey, 1965; Williams and Mount, 1965; and Wurtz, 1962).

Recommendation: The relationship between calcium and magnesium concentration, pH, and zinc toxicity is confusing and the separate effects have been little studied. Brungs (in press) has determined that 1/100 of the 96-hour TL_m value is a safe concentration for continuous exposure.

Copper: The same general considerations apply to the determination of safe levels of copper as apply to safe levels for zinc and the discussion of copper will be based on the same assumptions. From the published data, differences in species sensitivity to copper appear to be somewhat greater than for zinc (Anderson, 1950; Grande, 1966; Herbert and Vandyke, 1964; Jones, 1938; Lloyd, 1961b; Mount, in press; Pickering and Henderson, 1966a; Sprague, 1964a, b; Sprague and Ramsey, 1965; Trama, 1954; Turnbull, DeMann, and Weston, 1954). Mount (in press) has found that 1/30 of the 96-hour TL_m value is a safe concentration for continuous exposure of fish.

Bringmann and Kuhn (1959a, b) report that 0.15 mg/l copper is the threshold concentration which produces a noticeable effect on *Scenedesmus*. Maloney and Palmer (1956) report that 0.5

mg/l copper as copper sulfate produces the following percents of death in algae:

57 percent in 17 species of blue-green algae.
35 percent in 17 species of green algae.
100 percent in 6 species of diatoms.

Fitzgerald, Gerloff, and Borg (1952) report that 0.2 mg/l of copper (as copper sulfate) produces a 100-percent kill of *Microcystis aeruginosa.* Crance (1963) found 0.05 mg/l kills *Microcystis.* Hasrall (1962), working with *Chlorella vulgaris* found that 25 g/l of copper sulfate did not inhibit respiration if cultures were shaken. If shaking stopped, concentrations greater than 250 mg/l were toxic. Preliminary experiments indicate that lack of air increases toxicity of copper. Hale (1937)—according to Jordan, Day, and Hendrixson (1962)—reported that the following concentrations were necessary to control the indicated algae:

0.5 mg/l—*Cladophora.*
0.1 mg/l—*Hydrodictyon.*
0.12 mg/l—*Spirogyra.*
0.20 mg/l—*Ulothrix.*

Calcium and magnesium concentrations are usually not given for algal tests, but it would seem that the concentrations deemed safe for fish would also be acceptable for plankton.

Recommendation: The maximum copper (expressed as Cu) concentration (not including copper attached to silt particles or in stable organic combination) at any time or place should not be greater than $^1/_{10}$ the 96-hour TL_m value, nor should any 24-hour average concentration exceed $^1/_{30}$ of the 96-hour TL_m value.

Cadmium: Few studies have been made of the toxicity of cadmium in the aquatic environment. Mammalian studies have shown it to have substantial cumulative effects. Permissible levels in drinking water are 0.01 mg/l (USDHEW, 1962b), and concentrations of a few μg/g in food (McKee and Wolf, 1963) have caused sickness in human beings. Mount (1967) found accumulations in living bluegills as high as 100 μg/g (dry weight) and in the gills of dead catfish up to 1000 μg/g. Little accumulation was found in the muscle. Consideration should be given to acceptable residue levels in fish when establishing cadmium criteria.

Daphnia appears to be very sensitive to cadmium (Anderson, 1950). Bringmann and Kuhn (1959a) indicate *Scenedesmus,* and *Escherichia coli* are about equally sensitive. Data as yet unpublished (Pickering, in press) reveal that following prolonged exposure there is a large accumulation of cadmium in fish. Even though very little data are available yet, the evidence warrants a more restrictive requirement for cadmium than specified under the general bioassay section.

Recommendation: The concentration of cadmium must not exceed $^1/_{30}$ of the 96-hour TL_m concentration at any time or place and the maximum 24-hour average concentration should not exceed $^1/_{100}$ of the 96-hour TL_m concentration.

Hexavalent Chromium: The chronic toxicity of hexavalent chromium to fish has been studied by Olson (1958) and Olson and Foster (1956, 1957). Their data demonstrate a pronounced cumulative toxicity of chromium to trout and salmon. Mr. P. A. Olson (personal communication) of Battelle Memorial Institute advises that some recent comparisons of 48 and 96-hour TL_m concentrations with concentrations not adversely affecting the same species indicate that the application factor for hexavalent chromium is $^{16}/_{100,000}$ for salmon and $^{9}/_{100,000}$ for rainbow trout. He also feels however, that such factors are not valid for carp. Doudoroff and Katz (1953) found that bluegills tolerated a 45 mg/l level for 20 days in hard water. Cairns (1956), using chromic oxide (CrO_3), found that a concentration of 104 mg/l was toxic in 6 to 84 hours. *Daphnia* and *Microregma* exhibit threshold effects at hexavalent chromium levels of 0.016 to 0.7 mg/l.

Some data are available concerning the toxicity of chromium to algae. The concentrations of chromium that inhibit growth (Hervey, 1949) for the test organisms are as follows: Chlorococcales, 3.2 to 6.4 mg/l; Euglenoids, 0.32 to 1.6 mg/l; and diatoms, 0.032 to 0.32, mg/l. Chromium at sublethal doses sometimes stimulates algae. Patrick (unpublished data) has studied the effects of temperature on the toxicity of chromium to certain algae. Her findings on the concentrations which reduce population growth by 50 percent are as follows:

Nitzschia linearis.—50 percent reduction in growth of population as compared with control (soft water 44 mg/l Ca-Mg as $CaCO_3$)

22 C—0.208 mg/l Cr
28 C—0.261 mg/l Cr
30 C—0.272 mg/l Cr

Navicula seminulum var. hustedtii (hard water 170 mg/l Ca-Mg as $CaCO_3$)

22 C—0.254 mg/l Cr
28 C—0.343 mg/l Cr
30 C—0.433 mg/l Cr

Recommendation: Data are too incomplete to do more than urge caution in the discharge of chromium. Concentrations of 0.02 mg/l in soft water have been found safe for salmonid fishes.

TABLE III–5A. Pesticides *

INSECTICIDES

[48-hour TL_m values from static bioassay, in micrograms per liter. Exceptions are noted.]

Pesticide	Stream invertebrate[1] Species	TL_m	Cladocerans[2] Species	TL_m	Fish[3] Species	TL_m	Gammarus lacustris,[4] TL_m
Abate	Pteronarcys californica.	100			Brook trout	1,500	640
Aldrin[5]	P. californica	8	Daphnia pulex.	28	Rainbow trout.	3	12,000
Allethrin	P. californica	28	D. pulex	21	do	19	20
Azodrin					do	7,000	
Aramite			D. magna	345	Bluegill	35	100
Baygon[5]	P. californica	110			Fathead	25	50
Baytex[5]	P. californica	130	Simocephalus serrulatus.	3.1	Brown t.	80	70
Benzene hexachloride (lindane).	P. californica	8	D. pulex	460	Rainbow t.	18	88
Bidrin	P. californica	1,900	D. pulex	600	do	8,000	780
Carbaryl (sevin)	P. californica	1.3	D. pulex	6.4	Brown t.	1,500	22
Carbophenothion (trithion).			D. magna	0.009	Bluegill	225	28
Chlordane[5]	P. californica	55	S. serrulatus	20	Rainbow t.	10	80
Chlorobenzilate			S. serrulatus	550	do	710	
Chlorthion			D. magna	4.5			
Coumaphos			D. magna	1			0.14
Cryolite			D. pulex	5,000	Rainbow t.	47,000	
Cyclethrin			D. magna	55			
DDD (TDE)[5]	P. californica	1,100	D. pulex	3.2	Rainbow t.	9	1.8
DDT[5]	P. californica	19	D. pulex	0.36	Bass	2.1	2.1
Delnav (dioxathion)					Bluegill	14	690
Delmeton (systex)				14	do	81	
Diazinon[5]	P. californica	60	D. pulex	0.9	do	30	500
Dibrom (naled)	P. californica	16	D. pulex	3.5	Brook t.	78	160
Dieldrin[5]	P. californica	1.3	D. pulex	240	Bluegill	3.4	1,000
Dilan			D. magna	21	do	16	600
Dimethoate (cygon).	P. californica	140	D. magna	2,500	do	9,600	400
Dimethrin					Rainbow t.	700	
Dichlorvos[5] (DDVP)	P. californica	10	D. pulex	0.07	Bluegill	700	1
Disulfoten (di-syston)	P. californica	18			do	40	70
Dursban	Pteronarcella badia	1.8			Rainbow t.	20	0.4
Endosulfan (thiodan)	P. californica	5.6	D. magna	240	do	1.2	64
Endrin[5]	P. californica	0.8	D. pulex	20	Bluegill	0.2	4.7
EPH			D. magna	0.1	do	17	36
Ethion	P. californica	14	D. magna	0.01	do	230	3.2
Ethyl guthion[5]			D. pulex		Rainbow t.		
Fenthion	P. californica	30	D. pulex	4			
Guthion[5]	P. californica	8	D. magna	0.2	Rainbow t.	10	0.3
Heptachlor[5]	P. badia	4	D. pulex	42	do	9	100
Kelthane (dicofel)	P. californica	3,000	D. magna	390	do	100	
Kepone					do	37.5	
Malathion[5]	P. badia	6	D. pulex	1.8	Brook t.	19.5	1.8
Methoxychlor[5]	P. californica	8	D. pulex	0.8	Rainbow t.	7.2	1.3
Methyl parathion[5]			D. magna	4.8	Bluegill	8,000	
Morestan	P. californica	40			do	96	
Ovex	P. californica	1,500			do	700	
Paradichlorobenzene					Rainbow t.	880	
Parathion[5]	P. californica	11	D. pulex	0.4	Bluegill	47	6
Perthane			D. magna	9.4	Rainbow t.	7	
Phosdrin[5]	P. californica	9	D. pulex	0.16	do	17	310
Phosphamidon	P. californica	460	D. magna	4	do	8,000	3.8
Pyrethrins	P. californica	54	D. pulex	25	do	54	18
Rotenone	P. californica	900	D. pulex	10	Bluegill	22	350
Strobane[5]	P. californica	7			Rainbow t.	2.5	
Tetradifon (tedion)					Bluegill	1,100	140
TEPP[5]					Fathead	390	98
Thanite			D. magna	450			
Thimet					Bluegill	5.5	70
Toxaphene[5]	P. californica	7	D. pulex	15	Rainbow t.	2.8	70
Trichlorofon (dipterex).[5]	P. badia	22	D. magna	8.1	do	160	80
Zectran	P. californica	16	D. pulex	10	do	8,000	76

* See notes following Table III-5B.

Pesticides

A general description of the use and the effects of pesticides on aquatic life is given in the marine section. Basically, their effects are similar in both the marine and fresh water environments.

The addition of any persistent chlorinated hydrocarbon pesticides is likely to result in damage to aquatic life. Therefore, as concentrations of these chemicals increase in the aquatic environment, progressive damage will result. The acute effects usually will be recognized, but the chronic consequences may not be observed for some time.

The use of other kinds of chemical pesticides in or around fresh waters may produce a variety of acute and chronic effects on fish and the other components of the biota. Because these other chemicals are usually not as persistent as the chlorinated hydrocarbons, the Subcommittee feels some of them can be used around water, but only in amounts below those that produce chronic damage to desirable species.

Recommendation: (1) *Chlorinated hydrocarbons.*—Since any addition of persistent chlorinated hydrocarbon insecticides in likely to result in permanent damage to aquatic populations, their use should be avoided.

(2) *Other chemical pesticides.*—Addition of other kinds of chemicals used as insecticides, herbicides, fungicides, defoliants, acaracides, algicides, etc., can result in damage to some organisms. Table III–5 lists the 48-hour TL_m values for a number of pesticides for various types of fresh water organisms. To provide reasonably safe concentrations of these materials in receiving waters, application factors ranging from 1/10 to 1/100 should be used with these values depending on the characteristic of the pesticide in question and used as specified earlier in the section on application factors. Concentrations thus derived tentatively may be considered safe under the environmental conditions recommended.

Other Toxic Substances

Detergents and Surfactants: The toxicity of ABS has been reported by many workers. A wide range of endpoints have been used as criteria and while comparison is difficult, a reasonable conclusion is possible. There is no agreement on the effect of calcium and magnesium concentration. Recommendations are based on the data from table III–6.

Recommendation: With continuous exposure, the concentration of ABS should not exceed 1/7 of the 48-hour TL_m concentration. Concentrations as high as 1 mg/l may be tolerated infrequently for periods not exceeding 24 hours. ABS may increase the toxicity of other materials.

Much less work has been done on LAS, a newer, degradable detergent, than on ABS. Bardach, Fujiya, and Holl (1965) report that 10 mg/l is lethal to bullheads and 0.5 mg/l will erode 50 percent of the taste buds within 24 days. For fathead minnow fry, Pickering (1966) reports a 9-day TL_m of 2.3 mg/l. Thatcher and Santner (1967) report 96-hour TL_m values from 3.3 to 6.4 mg/l for five fish species. Swisher, O'Rourke, and Tomlinson (1954), testing bluegills, found TL_m values of 3 mg/l for LAS and 12 carbon homologs and 0.6 mg/l for 14 other carbon homologs. An intermediate degradation product had a TL_m of 75 mg/l. Dugan (1957) found that sensitivity to chlorinated pesticides possibly increased after exposure to detergent. Other studies as yet unpublished indicate a surprising increase in toxicity at low dissolved oxygen concentrations.

Pickering and Thatcher (in press), in the only reported study on reproduction, found that 0.6 mg/l had no measurable effect on reproduction or growth but 1.1 mg/l had an effect. In tests with five species of fishes. Thatcher and Santner (1967) found two species which were more sensitive to LAS than fathead minnows.

With both ABS and LAS detergents, the more readily degradable components are the more toxic. As a result, the components remaining will be less toxic than the original product.

Recommendation: The concentration of LAS should not exceed 0.2 mg/l of 1/7 of the 48-hour TL_m concentration, whichever is the lower.

Cyanide: Although it has been studied extensively, cyanide is not well understood as a hazard to aquatic life. Certain unique and peculiar characteristics necessitate special treatment of this chemical even though acceptable concentrations cannot be given.

Recent work on fish by Doudoroff et al. (1966), has demonstrated that HCN rather than CN is the toxic component. Except for certain extremely toxic heavy metals (silver, for example) the toxicity of metallo-cyanide complexes can also be attributed to the HCN. This then makes the effect of pH on cyanide toxicity of great importance. Doudoroff (1956) demonstrated a thousandfold increase in the toxicity of a nickelo-cyanide complex associated with a drop in pH from 8.0 to 6.5. A change in pH from 7.8 to 7.5 increases the toxicity ten times. The data reported by Cairns and Scheier (1963b) indicate that the calcium-magnesium concentrations (hardness) do not materially affect cyanide toxicity. It should be noted that in their test solutions while the calcium-magnesium concentration of their soft and hard

TABLE III-55. Pesticides, cont.

HERBICIDES, FUNGICIDES, DEFOLIANTS, ALGICIDES

Pesticide	Stream invertebrate[1] Species	Stream invertebrate[1] TL_m	Cladocerans[2] Species	Cladocerans[2] TL_m	Fish[3] Species	Fish[3] TL_m	Gammarus lacustris,[4] TL_m
Ametryne	------	------	------	------	Rainbow t.	3,400	------
Aminotriazole	------	------	------	------	------	------	------
Aquathol	------	------	------	------	Bluegill	257	------
Atrazine	------	------	Daphnia magna.	3,600	Rainbow t.	12,600	------
Azide, potassium	------	------	------	------	Bluegill	1,400	10,000
Azide, sodium	------	------	------	------	do	960	9,000
Copper chloride	------	------	------	------	do	1,100	------
Copper sulfate	------	------	------	------	do	150	------
Dichlobenil	Pteronarcys californica.	44,000	Daphnia pulex.	3,700	do	20,000	1,500
2,4-D, PGBEE	------	------	D. pulex	3,200	Rainbow t.	960	1,800
2,4-D, BEE	P. californica	1,800	------	------	Bluegill	2,100	760
2,4-D, isopropyl	------	------	------	------	do	800	------
2,4-D, butyl ester	------	------	------	------	do	1,300	------
2,4-D, butyl + isopropyl ester.	------	------	------	------	do	1,500	------
2,4,5-T isooctyl ester	------	------	------	------	do	16,700	------
2,4,5-T isopropyl ester	------	------	------	------	do	1,700	------
2,4,5-T PGBE	------	------	------	------	do	560	------
2(2,4-DP) BEE	------	------	------	------	do	1,100	------
Dalapon	P. californica Very low toxicity	------	D. magna	6,000		Very Low toxicity.	
Dead-X	P. californica	5,000	D. pulex	3,700	Rainbow t.	9,400	5,600
DEF	P. californica	2,300	------	------	Bluegill	36	230
Dexon	P. californica	42,000	------	------	Bluegill	23,000	6,000
Dicamba	------	------	------	------	non-tox.	------	5,800
Dichlone	------	------	D. magna	26	Rainbow t.	48	11,500
Difolitan	P. californica	150	------	------	Channel Cat.	31	6,500
Dinitrocresol	P. californica	560	------	------	Rainbow t.	210	------
Diquat	------	------	------	------	do	12,300	------
Diuron	P. californica	2,800	D. pulex	1,400	do	4,300	380
Du-ter	------	------	------	------	Bluegill	33	------
Dyrene	------	------	D. magna	490	------	15	------
Endothal, copper	------	------	------	------	Rainbow t.	290	------
Endothal, dimethylamine.	------	------	------	------	do	1,150	------
Fenac, acid	P. californica	70,000	------	------	do	16,500	------
Fenac, sodium	P. californica	80,000	D. pulex	4,500	do	7,500	18,000
Hydram (molinate)	P. californica	3,500	------	------	do	290	------
Hydrothol 191	------	------	------	------	do	690	1,000
Lanstan (korax)	------	------	------	------	do	100	5,500
LFN	------	------	------	------	do	79	------
Paraquat	P. californica Very low toxicity	------	D. pulex	3,700	Very low toxicity.	------	18,000
Propazine	------	------	------	------	Rainbow t.	7,800	------
Silvex, PGBEE	------	------	D. pulex	2,000	do	650	------
Silvex, isoctyl	------	------	------	------	Bluegill	1,400	------
Silvex, BEE	------	------	------	------	do	1,200	------
Simazine	P. californica	50,000	------	------	Rainbow t.	5,000	21,000
Sodium arsenite	P. californica Very low toxicity	------	Simocephalus serrulatus.	1,400	do	36,500	------
Tordon (picloram)	------	------	------	------	do	2,500	48,000
Trifuralin	P. californica	4,200	D. pulex	240	do	11	5,600
Vernam[5] (vernolate)	------	------	------	------	do	5,900	25,000

---

[1] Stonefly bioassay was done at Denver, Colo., and at Salt Lake City, Utah. Denver tests were in soft water (35 mg/l TDS), non-aerated, 60 F. Salt Lake City tests were in hard water (150 mg/l TDS), aerated, 48–50 F. Response was death.

[2] *Daphnia pulex* and *Simocephalus serrulatus* bioassay was done at Denver, Colo., in soft water (35 mg/l TDS), non-aerated, 60 F. *Daphnia magna* bioassay was done at Pennsylvania State University in hard water (146 mg/l TDS), non-aerated, 66 F. Response was immobilization.

[3] Fish bioassay was done at Denver, Colo., and at Rome, N.Y. Denver tests were with 2-inch fish in soft water (35 mg/l TDS), non-aerated; trout at 55 F; other species at 65 F. Rome tests were with 2–2½-inch fish in soft water (6 mg/l TA; pH 5.85–6.4), 60 F. Response was death.

[4] *Gammarus* bioassay was done at Denver, Colo., in soft water (35 mg/l TDS), non-aerated, 60 F. Response was death.

[5] Becomes bound to soil when used according to directions, but highly toxic (reflected in numbers) when added directly to water.

water was greatly different, the pH and toxicity were similar.

Burdick and Lipschuetz (1950) show that some metallo-cyanide complexes decompose in sunlight and become highly toxic due to release of cyanide from the complex. Cairns and Scheier (1963b) found some increase in toxicity at reduced oxygen concentrations and Henderson, et al. (1961) demonstrated marked cumulative toxicity of an organic cyanide in 30-day tests.

The toxicity of cyanide to diatoms varied little with change of temperature and was a little more

TABLE III–6. Effect of Alkyl-Aryl Sulfonate, Including ABS, on Aquatic Organisms

Organisms	Concentration (mg/l)	Time	Effect	References
Trout	5.0	26 to 30 hours	Death	Wurtz-Arlet, 1960.
	3.7	24 hours	TL_m	
	5.0		Gill pathology	Schmid and Mann, 1961.
Bluegills	4.2	24 hours	TL_m	Turnbull, et al., 1954.
	3.7	48 hours	TL_m	
	0.86		Safe	
	16.0	30 days	TL_m	Lemke and Mount, 1963.
	5.6	90 days	Gill damage	Cairns and Scheier, 1963.
	17.0	96 hours	TL_m	
Fathead minnows	2.3		Reduced spawning	Pickering, 1966.
	13.0	96 hours	TL_m	Henderson, et al., 1959.
	11.3	96 hours	TL_m	Thatcher, 1966.
Fathead minnow fry	3.1	7 days	TL_m	Pickering, 1966.
Pumpkinseed sunfish	9.8	3 months	Gill damage	Cairns and Scheier, 1964.
Salmon	5.6	3 days	Mortality	Holland, et al., 1960.
Yellow bullheads	1.0	10 days	Histopathology	Bardach, et al., 1965.
Emerald shiner	7.4	96 hours	TL_m	Thatcher, 1966.
Bluntnose minnow	7.7	96 hours	TL_m	Thatcher, 1966.
Stoneroller	8.9	96 hours	TL_m	Thatcher, 1966.
Silver jaw	9.2	96 hours	TL_m	Thatcher, 1966.
Rosefin	9.5	96 hours	TL_m	Thatcher, 1966.
Common shiner	17.0	96 hours	TL_m	Thatcher, 1966.
Carp	18.0	96 hours	TL_m	Thatcher, 1966.
Black bullhead	22.0	96 hours	TL_m	Thatcher, 1966.
"Fish"	6.5		Min. lethality	Leclerc and Devlaminck, 1952.
Trout sperm	10.0		Damage	Mann and Schmid, 1961.
Daphnia	5.0	96 hours	TL_m	Sierp and Thiele, 1954.
	20.0	24 hours	TL_m	Godzch, 1961.
	7.5	96 hours	TL_m	Godzch, 1961.
Lirceus fontinalis	10.0	14 days	6.7 percent survival (hard water).	Surber and Thatcher, 1963.
Crangonyx setodactylus[1]	10.0	14 days	0 percent survival (hard water).	Surber and Thatcher, 1963.
Stenonema ares	8.0	10 days	20–33 percent survival.	Surber and Thatcher, 1963.
	16.0	10 days	0 percent survival	Surber and Thatcher, 1963.
Stenonema heterotarsale	8.0	10 days	40 percent survival	Surber and Thatcher, 1963.
	16.0	10 days	0 percent survival	Surber and Thatcher, 1963.
Isonychia bicolor	8.0	9 days	0 percent survival	Surber and Thatcher, 1963.
Hydropsychidae (mostly cheumatopsyche).	16.0	12 days	37–43 percent survival.	Surber and Thatcher, 1963.
	32.0	12 days	20 percent survival	Surber and Thatcher, 1963.
Orconectes rusticus	16.0	9 days	100 percent survival	Surber and Thatcher, 1963.
	32.0	9 days	0 percent survival	Surber and Thatcher, 1963.
Goniobasis livescens	16.0	12 days	40–80 percent survival.	Surber and Thatcher, 1963.
	32.0	12 days	0 percent survival	Surber and Thatcher, 1963.
Snail	18.0	96 hours	TL_m	Cairns and Scheier, 1964.
	24.0	96 hours	TL_m	Cairns and Scheier, 1964.
Chlorella	3.6		Slight growth reduction.	Maloney, 1966.
Nitzchia linearis	5.8		50 percent reduction in growth in soft water.	Cairns, et al., 1964.
Navicula seminulum	23.0		50 percent reduction in growth in soft water.	Cairns, et al., 1964.

---

[1] Misidentified originally as Synurella.

toxic in soft water than in hard water (Patrick, unpublished data). For *Nitzchia linearis* a 50-percent reduction in growth of the population in soft water (44 mg/l Ca-Mg as $CaCO_3$) occurred as follows: 0.288 mg/l (CN) at 72 F, 0.295 mg/l at 82 F, and 0.277 mg/l at 86 F. For *Navicula seminulum* var. hustedtii, the concentrations that reduced growth of the population 50 percent in hard water (170 mg/l Ca-Mg as $CaCO_3$) were as follows: 0.356 mg/l at 72 F, 0.491 mg/l at 82 F, and 0.424 mg/l at 86 F.

Recommendation: Permissible concentrations of cyanide should be determined by the flow-through bioassay method described in the bioassay section. These tests should be conducted with DO, temperature, and pH at recommended levels for the factors under which the cyanide (HCN) is most toxic or under local water conditions at which it is the most toxic.

Ammonia: The toxicity of ammonia has been studied by several investigators but because of inadequate reporting and unsatisfactory experimental control, much of the work is not usable. Doudoroff and Katz (1950), Wuhrmann, et al. (1947), and Wuhrmann and Woker (1948) give a complete account of the pH effect on ammonia toxicity and demonstrate that toxicity is dependent primarily on undissociated NH_4OH and nonionic ammonia. They found no obvious relationship between time until loss of equilibrium and total ammonium content. They also demonstrated a striking synergy between ammonia and cyanide. McKee and Wolf (1963) state that toxicity is increased markedly by reduced dissolved oxygen. Field studies by Ellis (1940) and other observations lead to the conclusion that at pH levels of 8.0 and above total ammonia expressed as N should not exceed 1.5 mg/l. It has been found that 2.5 mg/l total ammonia expressed as N is acutely toxic.

Recommendation: Permissible concentrations of ammonia should be determined by the flow-through bioassay with the pH of the test solution maintained at 8.5, DO concentrations between 4 and 5 mg/1, and temperatures near the upper allowable levels.

Others: Especially significant sources of wastes that must be considered individually are derived from tar, gas, and coke-producing plants, pulp and paper mills, petroleum refining and petrochemical plants, waterfront boating activities, and special-purpose laboratories. These problems are discussed in the toxicity portion of the section on water quality requirements for marine organisms.

marine and estuarine organisms

ESTUARIES are recognized as being of critical importance in man's harvest of economically useful living marine resources. It is in these areas that the maximum conversion of solar energy into aquatic plant life takes place and they are justly identified as "nurseries" since so many animals utilize them for feeding their early life stages. Some species, such as the oyster, spend their entire life span in the estuary, while the shrimp and menhaden reside there only as juveniles. The salmon and a few others use the estuary primarily as a pathway. In sum, however, more than half of the over 4.5 billion pounds of fishery products harvested by U.S. fisherman annually is derived from animals dependent for their existence on clean estuarine waters during some part or all of their life cycle.

Pollution of estuarine and coastal waters is difficult to assess because of the special qualities of this environment. Technically, any foreign substance or environmental condition that interferes with a desired use may be considered a pollutant, but we are concerned with those substances present at high enough concentrations or environmental changes great enough to cause deleterious effect. Many naturally occurring substances in salt water become toxic when their concentrations are increased artificially or by natural processes.

The problem in establishing criteria in estuaries arises from the fluctuating nature of the water quality, both daily and seasonally, and geographically within the estuary. Changes in salinity, pH, turbidity, and temperature may alter greatly the critical toxic concentration of a pollutant. Most chlorinated hydrocarbon pesticides, for example, are significantly more acutely toxic at summer rather than winter water temperatures and at least one of the common detergents becomes decidedly more toxic to fish as salinity levels increase.

The most obvious effect of tidal action in the estuary is to change water depth. This indirectly changes current patterns, water temperature, and the density of motile animal populations. Depending on the geography of the estuary and the amount of fresh water drainage into it, salinity patterns may vary from relatively uniform conditions throughout a tidal cycle to situations in which the water is clearly stratified with a layer of relatively fresh water overlying the bottom salt water, or to situations in which the major portion of the water mass changes from fresh to salt and back to fresh again.

In shallow, broad estuaries, wind may be the dominant factor in causing water movements which change salinity and temperature patterns. The volume of fresh water discharged into an estuary may be a major factor in establishing coastal currents that transport pollution loads from one region to another.

We are dealing, then, with an environment in which the characteristics of the receiving water are usually fluctuating, frequently unexpectedly. As a result, its ability to dilute and disperse a burden of toxicants is unpredictable without detailed local investigations.

Pollution in the estuary may be derived from contamination hundreds of miles upstream in the river basin or it may be of purely local origin. Silt plays a major role in the transport of toxicants, especially pesticides, down to the estuary. Agricultural chemicals are adsorbed on silt particles. Under poor farming practices, as much as 11 tons of silt per acre per year may be washed by surface water into a drainage basin. Surface mining and deforestation further accelerate the process of erosion and permit the transport of terrestrial chemical deposits to the marine environment.

Atmospheric drift is also an important factor in the transport of pollutants to the aquatic environment (Cohen and Pinkerton, 1966). Much of the tonnage of aerially applied pesticides fails to reach the designated spray areas and the presence of 5 μg/l of DDT in presumably untreated Alaskan rivers indicates the magnitude of this facet of the pollution problem. The continuous presence of 5 μg/l of DDT in the marine environment would decrease the growth of oyster populations by nearly 50 percent.

Toxic pollutants may be passed directly into the marine environment as contaminants of industrial and domestic waste effluents or they may be intentionally placed there as in the control of various noxious insects by spraying marsh and littoral habitats with synthetic pesticides. Experimentally, some synthetic insecticides have been applied directly to estuarine bottoms in efforts to control oyster pests.

Finally, there are naturally occurring substances such as lignins and phosphate compounds which in times of flood may be carried to the estuary in sufficient quantity to constitute a pollution hazard.

Salinity

The spatial and temporal distribution of salinity profoundly affects the activities of many estuarine species in tidal tributaries (Andrews, 1964; Emery and Stevenson, 1957; Hargis, 1965 and 1966; Pearse and Gunter, 1957; Pritchard, 1953). Some bottom organisms, e.g., *Crassostrea virginica*, are able to survive lower salinities than can their predators and disease-causing organisms. Hence, in some tidal tributaries, oysters thrive in regions where they are sheltered from these pests by low salinity. Natural alterations in salinity distribution have been reportedly followed by increased mortality of oysters. It is clear that care must be exercised in the approval of engineering projects or industrial processes that will alter salinity regimes in tidal tributaries and lagoons and in their associated wetlands.

Salinity patterns can be caused to vary from "normal" by alterations in character of freshwater inflow and basin geometry. These are the same factors that produce changes in circulation. In fact, salinity alterations are precursors to changes in density currents.

Recommendation: For the protection of estuarine organisms, no changes in channels, in the basin geometry of the area, or in fresh water inflow should be made that would cause permanent changes in isohaline patterns of more than ±10 percent of the natural variation.

Currents

Despite their large volumes, tidal waters, especially those in tributaries of the seas, have special circulatory characteristics that may affect their ability to assimilate wastes. For example, tidal action slows the already slowed (due to lowered slope and resulting reduced speed of gravity-induced flow) seaward movement of water in tidal rivers and streams. This alternate up and down stream movement of the water in the freshwater portion of the tidal tributary is confounding enough in itself (Ketchum, 1950 and 1951; Stommel, 1953a, b) but in the estuarine reach, the area where sea salts are noticeable, further complexities often occur (Bowden, 1963; Hargis, 1965; Redfield, 1951). In horizontally and vertically stratified mixing estuaries, there are two streams. The upper stream, fresher and lighter, has a net-flow downstream while the lower stream, saltier and heavier, flows inward or upstream. Since these surface currents and bottom counter-currents often extend far to sea off the mouths of large tidal tributary or estuarine systems, as well as far upstream, significant upstream transport of materials in solution or suspension in the counter-current can occur. These circulatory features are important in the life cycles of many estuarine species. For example, oyster and barnacle setting is related to tidal and nontidal currents (Barlow, 1955; Bousfield, 1955; Emery and Stevenson, 1957; Hargis, 1966; Ketchum, 1954; Pritchard, 1953). Large disturbances of current patterns can disrupt the life cycles of estuarine organisms. Hence, projects that alter current patterns should be carefully evaluated and controlled.

It is possible to alter circulatory patterns in tidal tributaries by (1) changing the quantity, timing, and location of fresh water inflow, (2) changing the geometry of the basin. The former can be accomplished by construction and operation of reservoirs above or below the fall line (defined as the uppermost limit of ocean's tidal activity). The latter can be accomplished by shoreline or bottom modification; e.g., drainage, bulkheading and filling, channel dredging, and subaqueous spoil disposal or mining. Oyster harvesting practices have been known to produce marked changes in bottom geometry (Hargis, 1966).

Recommendation: In view of the requirements of estuarine organisms and the nature of marine waters, no changes in basin geometry or fresh water inflow should be made in tidal tributaries which will alter current patterns in such a way as to cause adverse effects.

pH

Despite the great emphasis given to the importance of pH in the literature, little is known of its direct physiological effects on marine organisms. Its indirect effects, however, are extremely significant. Even a slight change in pH indicates that the buffering system inherent in sea water has been altered radically and that either a potential or actual carbon dioxide imbalance exists. This imbalance can be deleterious or disastrous to marine life. A second indirect effect is that pH can influence the toxicity of other materials. Cyanide and ammonia, discussed under "Toxicity," are outstanding examples of this kind of action.

Recommendation: Materials that extend normal ranges of pH at any location by more than ±0.1 pH unit should not be introduced into salt water portions of tidal tributaries or coastal waters. At no time should the introduction of foreign materials cause the pH to be less than 6.7 or greater than 8.5.

Temperature

Temperature requirements of marine and estuarine organisms in the biota of a given region may vary widely. Therefore, if we are to maintain temperature favorable to the biota, all important species, including the most sensitive, must be protected. It has been found that organisms in the intertidal zone vary considerably in their ability to withstand high temperatures. Those in the uppermost areas of the tidal zone generally can withstand higher temperatures than those in the lower portions of the tidal zone and these in turn generally can withstand higher temperatures than the same species of animals living in the subtidal zones. In addition, when considering the coastline as a whole, we must recognize that there are various races within a given species which may vary considerably in their environmental requirements, or in their ability to withstand extreme conditions.

In our marine waters, there is a great mixture of species. Species typical of higher latitudes are found with species that are more abundant farther south. Tropical or subtropical species generally will spawn in the summer months. Species from the higher latitudes require low water temperature for spawning and the development of the young. Thus, they usually spawn in the winter months and temperatures at that time are critical. Any warming of the water during the cold weather or winter period could be disastrous from the standpoint of the elimination of the more northerly species. In some instances, a rise in winter temperatures of only 2 or 3 F might be sufficient to prevent spawning and thus eliminate these species from the biota.

In the northern portions of the country there is generally a great range in natural temperatures. In southern areas, as we approach the tropics, we find smaller overall temperature ranges. In the tropics or subtropics, optimum temperatures for many forms are only a few degrees lower than maximum lethal temperatures. Great care should be exercised, therefore, to prevent harmful increases in maximum summer temperatures in tropical areas.

In general, temperatures in the marine waters do not change as rapidly nor do they have the overall range from extreme to extreme as they do in fresh waters. Marine and estuarine fishes, therefore, are less tolerant of temperature variation. They can accommodate somewhat, but overall temperature range and rate of change are even more important here than they are in fresh waters. It has been observed that when surface water temperatures over the Georges Bank increased from 46 to 68 F, the larval fish died at 65 F. It has been found that species in subtropical and tropical environments are living at temperatures that are only a few degrees less than their lethal temperatures. In the most northern forms, extensive variations in seasonal temperatures are a necessity for orderly development and growth. Spawning and development frequently occur at lower temperatures and the sexual products ripen on rising temperatures after a period of low temperatures. Temperatures above or below the optimum range may delay or speed up development. They may inhibit swimming ability and the effectiveness of food utilization may be decreased with increasing temperatures in the upper viable range. Fishes and other forms are also more susceptible to parasites and diseases at temperatures outside of their optimum range. In regard to rapid changes in temperature, it has been found that a drop in temperature from 58 to 43 F kills sardines. Tolerable temperature minima vary with the population and its past temperature history. Kills have occurred off the Texas coast at 40 F whereas kills of the same species have occurred off Bermuda at a drop to 59 F. Many kills have occurred in nature due to unusually low temperatures. Kills also occur due to natural high temperatures. Yellowtail flounder and whiting larvae died when they drifted from an area of 44 F to one of 64 F. It has been reported that 61 F is best for the developing of mackerel, but 70 F is too high. These are merely illustrations of what might happen to species occurring in inshore waters.

It is apparent from the foregoing that data are very sparse on temperature requirements of marine and estuarine species. It is very difficult, therefore, to attempt to suggest temperature requirements for marine and estuarine forms. The difficulty is compounded by the great extent of the Nation's shorelines, the differing natural temperature variations from north to south, and the geographic overlapping of species native to different latitudes. Consideration must be given to maximum allowable temperatures for both the summer period and the winter period.

In attempting to establish permissible levels of temperature increase in receiving waters due to heated waste discharges, precaution must be taken to prevent—

(a) excessive incremental increases above background values even though such incremental increases lie below maximum limits, and

(b) exceeding maximum natural background limits.

Such precautions are necessary to prevent gradual net increases in background temperatures due to the continuously increasing volumes of heated wastes being discharged into receiving waters.

The discharge of heated wastes into estuaries and other tidal tributaries must be managed so that no barrier to the movement or migration of fish and other aquatic life is created.

Recommendation: In view of the requirements for the well-being and production of marine organisms, it is concluded that the discharge of any heated waste into any coastal or estuarine waters should be closely managed. Monthly means of the maximum daily temperatures recorded at the site in question and before the addition of any heat of artificial origin should not be raised by more than 4 F during the fall, winter, and spring (September through May), or by more than 1.5 F during the summer (June through August). North of Long Island and in the waters of the Pacific Northwest (north of California), summer limits apply July through September, and fall, winter, and spring limits apply October through June. The rate of temperature change should not exceed 1 F per hour except when due to natural phenomena.

Suggested temperatures are to prevail outside of established mixing zones as discussed in the section on zones of passage.

Dissolved oxygen

Dissolved oxygen requirements of marine organisms are not as well known as those for freshwater forms. Studies have been made indicating that minimum dissolved oxygen concentrations of 0.75 to 2.5 are required for test species to resist death for 24 hours. Most marine species died when the dissolved oxygen dropped below 1.25 mg/l for a few hours. Reduced swimming speed and changes in blood and serum constituents occurred at dissolved oxygen levels of 2.5 to 3 mg/l. It was found that DO levels between 5.3 and 8 mg/l were satisfactory for survival and growth. Levels above 17 mg/l, however, produced adverse effects. Large fluctuations in dissolved oxygen from 3 to 8 mg/l, diurnal or otherwise, produced significantly more physiological stress in fishes than fluctuations from 3 to 6 mg/l. In tests made to date, it has been found that 5 to 8 mg/l of DO is apparently sufficient for all species of fish for good growth and general well being. It is generally recognized that in deeper waters DO values are often considerably less than 5.0 mg/l. In estuaries where there is a reduction in salinity, levels may drop to as low as 4 mg/l at infrequent intervals and for limited periods of time. It is probable that many marine animals can live for long periods of time at much lower levels of DO. Experimental studies with freshwater organisms have demonstrated, however, that low concentrations of DO at which adult fishes can live almost indefinitely, can inhibit feeding and growth. In determining DO requirements, it is essential to consider growth, reproduction, and other necessary life activities.

Recommendation: For the protection of marine resources, it is essential that oxygen levels shall be sufficient for survival, growth, reproduction, the general well-being, and the production of a suitable crop. To attain this objective, it is recommended that dissolved oxygen concentrations in coastal waters, estuaries, and tidal tributaries of the Nation, including Puerto Rico, Alaska and Hawaii, should be as follows:

(1) Surface dissolved oxygen concentrations in coastal waters shall not be less than 5.0 mg/l, except when natural phenomena cause this value to be depressed.

(2) Dissolved oxygen concentrations in estuaries and tidal tributaries shall not be less than 4.0 mg/l at any time or place except in dystrophic waters or where natural conditions cause this value to be depressed.

The committee would like to stress the fact that, due to a lack of fundamental information on the DO requirements of marine and estuarine organisms, these requirements are tentative and should be changed when additional data indicate that they are inadequate.

Crude oil and petroleum products

The discharge of crude oil and petroleum products into estuarine and coastal waters presents special problems in water pollution abatement. Oils from different sources have highly diverse properties and chemistry. Oils are relatively insoluble in sea and brackish waters and surface action spreads the oil in thin surface films of variable thickness, depending on the amount of oil present. Oil, when adsorbed on clay and other particles suspended in the water, forms large, heavy aggregates that sink to the bottom. Additional complications arise from the formation of emulsions in water, leaching of water soluble fractions, and coating and tainting of sedentary animals, rocks, and tidal flats.

Principal sources of oil pollution are numerous. Listed in order of their destructiveness to ecosystems, they are:

(1) Sudden and uncontrolled discharge from wells towards the end of drilling operation.
(2) Escape from wrecked and submerged oil tankers.
(3) Spillage of oil during loading and unloading operations, leaky barges, and accidents during transport.
(4) Discharge of oil-contaminated ballast and bilge water into coastal areas and on the high seas.
(5) Cleaning and flushing of oil tanks at sea. On the average, a ship's content of such wastes is estimated to contain 2 to 3 percent oil in 1,000 to 2,000 tons of waste.
(6) Spillage from various shore installations, refineries, railroads, city dumps, garages, and various industrial plants.

Spillage From Wrecked Oil Tankers

Even though wrecks of oil tankers along the Atlantic coast and subsequent spillage of oil into the sea have been reported several times, no thorough examination has been made of the effect of oil pollution on local marine life, except for frequent references to the destruction of waterfowl. One of these disasters attracted the general attention of the public and members of the Audubon Society of New England. One night in 1952, two tankers, the *Fort Mercer* and the *Pendleton*, went aground on the shoal of Monomoy Point, Cape Cod. Large amounts of oil spilled from the broken vessels, spread long distances along the shore, and were responsible for high mortality of ducks (scoters and eiders). Many thousands of oil-smeared dying birds were seen along the coast. Attempts to save some of the birds by removing the oil with various solvents failed. No published records are found on the effect of this massive spillage on aquatic life. According to the records of the Massachusetts Audubon Society, serious oil spreads threatening fish and bird life have occurred at least six times since 1923 along the beaches of Cape Cod. The latest occurrence was on Sunday, April 16, 1967. Heavy films of crude oil appeared along the coast from Chatham to Provincetown, Mass. and spread to Cape Cod Bay, Nantucket Island, and Boston. The shores of the National Seashore Park were seriously affected and hundreds of ducks and brant were found dead or dying.

The massive spillage of oil may constitute a disaster of a national and even international magnitude as has been dramatically demonstrated by the wreck in March 1967 of the super tanker *Torrey Canyon* carrying 118,000 tons of crude oil. About one-half of the load gradually spilled near Seven Stones Reef, off the southern coast of England, where the tanker was stranded. By the middle of April, patches of crude oil began to appear on the French coast in Brittany, threatening the productive oyster farms in the inlets and estuaries. It is obvious that a disaster of such magnitude is beyond the scope of an ordinary pollution problem in coastal waters. The probability of a recurrence of heavy oil spillage is, however, very real because of the present trend in the methods of transporting oil in very large and apparently vulnerable tankers. It has been reported that Japan operates a tanker, *Idemitsu Maru*, of 205,000 tons holding capacity. A super tanker of over 300,000 tons capacity is under consideration and a design of a 500,000-ton tanker appeared in the press.

Effect of Oil Spillage on Aquatic Life of a Small Marine Cove

W. J. North, et al. (1965) made a valuable study of the effect of massive spillage of crude oil into a small cove in lower California Bay. Prior to the spillage, the investigators were engaged in a study of bottom fauna and flora of the cove and were in possession of background information which made it possible for them to record the changes that took place after the water of the cove was contaminated by the 59,000 barrels of oil that escaped from the wreck of the tanker *Tampico* on March 29, 1957. Among the many dead and dying species observed a few weeks after the disaster, the most frequently found were abalones (*Haliotis fulgens, H. rufescens,* and especially *H. cracherodii*), lobsters (*Panulirus interruptus*), pismo clams (*Tivela stultorum*), mussels (*Mytilus sp.*), sea urchins (*Strongylocentrotus franciscanus, S. purpuratus*), and sea stars (*Pisaster giganteus, P. ochraceus*). A slight improvement of the bottom fauna was noticeable a few months after the disaster, but extensive recovery became apparent only 2 years later. Four years after the accident, the populations of abalones and sea urchins still were reduced greatly and seven species of animals previously recorded in the cove had not been found at all.

Combined Effect of Oil and Sewage Pollution

The oil and sewage pollution effects on aquatic organisms of the Novorossiyak Bay (Black Sea, U.S.S.R.) was recently studied by Kalugina, et al. (1967). For a number of years, this bay has been receiving a mixed daily discharge of 15,000 to 30,000 cubic meters of petroleum refinery wastes and domestic sewage. There is marked decrease of various valuable species of mollusks (*Spisula subtruncata, Tapes rugatus, Pecten ponticus*) and complete destruction of oyster beds (*Ostrea taurica*) due to the combined effect of pollution and depradations by a carnivorous gastropod (*Rapana*). Samples were collected 1 to 25 meters from the outfall for bioassay. Copepods (*Acartia clausi*) placed in samples taken 25 meters from the outfall were killed in 24 hours. Larvae of decapods and gastropods in samples taken 10 to 25 meters out perished in 3 to 4 days. *Calanus* was killed in 5 days in samples taken 1 meter out, but survived the 10-day test in the samples taken 5, 10, and 25 meters from the outfall. There also was a noticeable change in the distribution and species composition of benthic algae.

Color of Oil Film on the Surface of Water

The color of the oil film on the water surface is indicative of the thickness of the slick and may be used as an indicator of the volume of oil spilled.

According to data published by the American Petroleum Institute (1949), the first trace of color that may be observed as a surface film on the sea is formed by 100 gallons of oil spread over 1 square mile. Films of much darker colors may indicate 1,332 gallons of oil per square mile. Experiments conducted by the Committee on the Prevention of Pollution of the Seas (1953) showed that 15 tons of oil covered an area of 8 square miles. In 8 days, it had drifted about 20 miles from the point of discharge. The same committee (1953) indicated another source of oil pollution that should not be neglected. It has been found that unburned fuel oil escaping through the funnels of oil-burning ships may comprise 1 to 2 percent of the total oil consumed and it may be deposited on the sea surface. British investigators attributed the disappearance of eel grass (*Zostera*) to minute quantities of oil. Oil weakens the plant and makes it susceptible to attacks of a parasitic protozoan (*Labyrinthula*). Observations made several years ago at Woods Hole showed that young *Zostera* that began to reappear in local bays after several years of absence were already infected by this microorganism even though they appeared to be healthy.

Adsorption of Oil by Sand, Clay, Silt, and Other Suspended Particles

Oil of surface films is easily adsorbed on clay particles and other suspended materials, forming large and relatively heavy aggregates that sink to the bottom. The surface of the water may appear free from pollution, until the sediment is stirred by wave action and the released oil floats up again.

During World War II, a product known as "carbonized sand" was manufactured for the U.S. Navy and used for the primary purpose of rapidly removing oil spilled or leaked from ships. Carbonized sand was used principally as a rapid method to prevent and stop fires. Sand and oil aggregates, being much heavier than sea water, sank very rapidly and remained on the bottom. Experimental work has shown that the toxic effect of oils is not diminished by this method (Chipman and Galtsoff, 1949). Since the end of World War II, a number of preparations to be used as solvents, emulsifiers, and dispersing agents of oil slicks in harbor waters appeared in New Zealand, Western Europe, and the United States. These preparations are being offered under various trade names and their chemical composition is not always stated. It is often claimed that such compounds remove oil slicks more efficiently than mopping with straw or coarse canvas fabric (skrim), a method extensively used in Auckland Harbor (Chitty, 1948). It is, however, generally recognized that various detergents and emulsifiers are toxic to aquatic life and therefore compound the danger of oil pollution. Mechanical means such as preventing the spread of a slick by surrounding it with floating barriers (plastic booms), spreading sawdust and removing an oil aggregate by scooping or raking, and erecting grass or straw barriers along the beaches are probably more effective at present than the chemical methods of dispersing or dissolving oil. Even anchoring oil by combining it with relatively heavy carbonized sand seems to be preferable to chemical methods.

Toxicity of Crude Oil and Petroleum Products

Oil may injure aquatic life by direct contact with the organism, by poisoning with various water soluble substances that may be leached from oil, or by emulsions of oil which may smear the gills or be swallowed with water and food. A heavy oil film on the water surface may interfere with the exchange of gases and respiration.

A number of observations have been recorded of the concentrations of oil in sea water which are deleterious to various species. Experimental data, however, are scarce and consequently the toxicology of oil to marine organisms is not well understood.

Nelson (1925) observed marine mollusks (*Mya arenaria*) being destroyed by oil on tidal flats of Staten Island, N.Y. The Pacific coast sea urchin, *Strongylocentrotus purpuratus*, dies in about 1 hour in a 0.1 percent emulsion of diesel oil. After 20 to 40 minutes in this concentration the animals fail to cling to the bottom and may be washed away (North, et al., 1964).

Crude oil absorbed by carbonized sand does not lose its toxicity. This has been shown by laboratory experiments conducted at Woods Hole (Chipman and Galtsoff, 1949). The amount of oil used was limited to the quantity held in the sand, hence no free oil was present in the water. The oil-sand aggregates were placed in containers filled with sea water but never came into contact with the test animals. Four species were bioassayed: the very hardy toadfish (*Opsanus tau*) in the yolk sac stage, the moderately tolerant barnacle (*Balanus balanoides*), and oyster (*Crassostrea virginica*), and the extremely sensitive hydrozoan, (*Tubularia crocea*).

The survival of toadfish embryos was indirectly proportional to the concentration of oil in water.

In a concentration of 0.5 percent, the embryos survived for 13 days (end of test); in 1.25 percent, 8½ days; in 2.5 percent, 6 days; and in 5 percent, 4½ days. Barnacles suffered 80 to 90-percent mortality within 70 hours in 2.0-percent mixtures of oil in sea water. They became inactive in 23 hours in concentrations of 2 percent and above. *Tubularia* suffered 90 to 100-percent mortality within 24 hours after being placed in water containing a 1:200 oil-carbonized sand aggregate. Water extracts of crude oil were lethal within 24 hours at concentrations of 500 mg/l and greater.

Experiments with oysters consisted primarily of determining the effect of oil adsorbed on carbonized sand on the number of hours the oysters remain open and feeding and on the rate of water transport, across the gills. A paste-like aggregate of oil in carbonized sand (50 ml crude oil to 127 g sand) was prepared, wiped clean of excess oil, and placed in the mixing chamber. Sea water was delivered through this chamber to the recording apparatus at a rate slightly in excess of the rate of water transport by oyster gills (Galtsoff, 1964; Chipman and Galtsoff, 1949). There was a noticeable decrease in the number of hours the test oysters remained open and in the daily water transport rate through the gills. The time open was reduced from 95 to 100 percent during the first 4 days of testing to only 19.8 percent on the 14th day. The total amount of water transported per day, and presumably used for feeding and respiration, was reduced from 207 to 310 liters during the first 6 days to only 2.9 to 1 liter per day during the period between the eighth and 14th day of continuous testing. These tests indicate that oil incorporated into the sediments near oyster beds continues to leach water-soluble substances which depress the normal functions of the mollusk.

Critical observations are lacking on the effect of oil on pelagic larvae of marine invertebrates, but there is good reason to assume that crude oil and petroleum products are highly toxic to free-swimming larvae of oysters. Speer (1928) considers that they are killed by contact with surface oil film. Laboratory experience of Galtsoff (unpublished records) shows that oyster larvae from 5 to 6 days old were killed when minor quantities of fuel oil were spilled by ships in the Woods Hole harbor and the contaminated water penetrated into the laboratory sea water supply.

The tests described above leave no doubt that water-soluble substances are leached from oil spilled into water and adversely affect marine life. It is reasonable to assume that the water soluble materials of oil may contain various hydrocarbons, phenols, sulfides, and other substances toxic to aquatic life. The water-soluble fraction leached from crude oil is easily oxidized by aeration and loses its toxicity (Chipman and Galtsoff, 1949).

Carcinogenic Substances From Oil-Polluted Waters

Presence of hydrocarbons similar to benzopyrene in oil-polluted coastal waters and sediments of France in the Mediterranean was reported by Mallet (1965) and Mallet and Sardou (1965). The effluents from the industrial establishments on the shores at Villefranche Bay comprise tar substances, which contain benzopyrenes, benzo-8, 9-fluoranthene, dibenzanthracenes, chrysene, 10-methyl anthracene, and nitrogenous derivatives such as dimethylbenzacridine. These substances are carried out into the bay water and settle on the bottom. The pollution is augmented by incompletely burned oils discharged by turbine ships. The content of benzopyrene in bottom sediments ranges from 500 micrograms in 100 g sample collected at the depth of 8 to 13 cm to 1.6 micrograms at 200 cm. Similar contamination is of importance in the Gulf of Fos, Etang de Berne, and in the delta of the Rhone River.

Carcinogenic hydrocarbons were found to be stored in plankton of the bay of Villefranche, in concentrations varying from 2.5 to 40 micrograms per 100 g. Fixation of benzopyrenes was found also in the bodies of holothurians (Laiou, 1965) in a bay near Antibes. The reported concentration in the visceral mass of holothurian was slightly higher than that in the bottom sediment.

Observations on storage of carcinogenic compounds found in oil-polluted water are biologically significant. The important question of biological magnification as these compounds are ingested by plankton feeders remains unanswered and needs to be investigated.

Sampling of Oil-Polluted Sea Water

The question of the minimal concentration of oil and petroleum products consistent with uninhibited growth and reproduction of aquatic species is more difficult to answer than it is in the case of other contaminants. As has been shown above, oil is found in water in four distinct phases: (1) surface oil film, (2) emulsion in sea water, (3) extract of water soluble substances, and (4) semisolid aggregate of oil and sediment covering the bottom. Obviously, no single sample could include all four phases and the method of sampling should vary accordingly. Collection of samples of a heavy oil slick near the origin of spillage presents no particular difficulty because an adequate quantity may be scooped easily and placed in a proper container. Serious difficulty arises, however, in case of an irridescent film of oil approaching the thickness of a monomolecular layer. Garrett (1964), made a special study of slick-forming materials naturally occurring on sea surfaces and demonstrated their highly complex composition. The collection of very thin layers of surface water was made by means of a specially constructed plastic screen. The entrapped compounds were washed off into a large container (Garrett, 1962). He found surface-acting substances in all areas where the sea surface was altered by monomolecular films and concluded that "a chemical potential exists whereby such surface alterations can occur when conditions are suitable for the adsorption and compression of the surface-active molecules at the air/water boundary." The oil film at the air/water boundary may be composed of several interacting organic compounds. This complexity must be kept in mind in studies of oil pollution in sea water.

If a relatively thick layer of contaminated water is needed, the sample may be scooped or sucked from an area of sea surface enclosed by a floating frame. Interference due to wave ripples is minimized in this way.

For analysis of an oil emulsion in sea water, a sample of a desired volume may be collected by pump or by any type of self-closing water bottle lowered within the surf area.

For obtaining water soluble substances leached from oil sludge, sampling should be made by pumping or by using a water sampler lowered as close as possible to the oil-covered bottom.

Samples of oil adsorbed on sediments can be obtained by using bottom samplers designed to take quantitative samples.

Contamination of beaches by floating tar ballast and cleaning water discharged by ships sailing along our coast is of such common occurrence that at present it is almost impossible to find a public beach free from this nuisance. Cakes of solidified oil tar can be picked by hand from the tidal zone of any beach along the Atlantic and Gulf coasts.

Recommendation: Until more information on the chemistry and toxicology of oil in sea water becomes available, the following requirements are recommended for the protection of marine life. No oil or petroleum products should be discharged into estuarine or coastal waters in quantities that (1) can be detected as a visible film or sheen, or by odor, (2) cause tainting of fish and/or edible invertebrates, (3) form an oil-sludge deposit on the shores or bottom of the receiving body of water, or (4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity and color

Turbidity, color, and transparency are closely interrelated phenomena in water. They must be observed simultaneously because transparency is a function of turbidity, water color, and spectral quality of transmitted light. For practical purposes, however, it is more convenient to discuss them separately.

Turbidity

By observing the turbidity of sea water it is possible to determine the depth of the euphotic zone; i.e., the depth in which organic carbon is produced. Various particles suspended in water reduce the intensity of light by absorption and scattering. In the sea, the maximum depth of growth of attached plants varies. It is 160 m in the Mediterranean, 30 m in Puget Sound, and 10 m off Cape Cod. In general, benthic plants will not grow at a depth at which the light intensity is less than 0.3 percent of its surface value (Clarke, 1954). In any environment, the rate of photosynthesis decreases with the attenuation of light but the respiration rate remains approximately the same. Because the role of phytoplankton in organic production is far more important quantitatively than that of benthic plants, an increase in the turbidity of water diminishes primary productivity of the ocean biomass as indicated by the rate of growth of various planktonic algae.

For each species of plant, a level of light intensity may be reached at which the rate of photosynthesis becomes equal to the rate of respiration. This level is designated as compensation intensity and the depth at which this value is found is called the compensation depth. For marine phytoplankton, it has been determined that compensation intensity is about 100 ft-candles, or 1 percent of the value of full sunlight (Clarke, 1954). In natural waters, the compensation depth varies; e.g., in the Gulf of Maine it was found to be 30 m while at Woods Hole only 7 m.

In many coastal waters, the principal cause of turbidity is the discharge of silt carried out by the principal rivers. Secchi disc readings show that the transparency of water at the mouths of large rivers during flood stage may be reduced to a few centimeters. At normal river stages, the disc may be visible at several meters below the surface. Observations from an airplane are useful in recording the distribution of brackish, silt-laden waters along the coast. Silting of the estuaries and adjacent coastal water should be considered as a special case of pollution resulting from deforestation, overgrazing and faulty agricultural practices, road construction, and other land management abuses.

Mixed effluents from various industrial plants and domestic sewage increase the turbidity of receiving water. It is difficult to distinguish between the effect of the attenuation of light due to suspended particles and the direct effect of the particles in suspension on the growth and physiology of aquatic organisms. Natural silt taken from the bottom of the sea and kaolin affect the development of eggs and the growth of larvae of oysters and hard shell clams (*Mercenaria mercenaria*). In a suspension of 2 g of dry silt in a liter of sea water, only 39 percent of oyster larvae completed development. In 3 g per liter there was no development (Loosanoff, 1962). Growth of *Mercenaria* clams was retarded in the concentration of 1 to 2 g/l, but appeared to be normal at 0.75 g/l. Development was completely suppressed in the concentration of silt from 3 to 4 g/l (Davis, 1960). Silt concentration of 0.1 g/l caused a 57-percent decrease in the water transport of an adult oyster. In 4 g/l, the depression was 94 percent (Loosanoff, 1962). The turbidity used in these experiments probably is equivalent to 750 to 4,000 mg/l of turbidity standards, although direct comparison of figures cannot be made accurately.

The principal significance of turbidity observations in a study of pollution is the determination of the depth of the euphotic zone as a factor affecting primary productivity of the sea (Ryther, 1963). Determination of the coefficient "k" defined as the natural logarithm of the fraction of incident light penetrating to a given depth is of great importance in studies of organic production. In the temperate and northern parts of the ocean, values of "k" range between 0.10 to 0.20 and correspond to depths of 50 to 25 m. In more turbid coastal waters, the coefficient of extinction is as high as 1.0 and a compensation depth of 5 m is commonly encountered. These values may be used as a basis for comparing the characteristics of uncontaminated waters with those of highly turbid and polluted waters of coastal and inshore areas. A considerable part of the turbidity of these areas is attributable to nonliving particles.

It must remembered, also, that very high turbidity of sea water may be due entirely to blooms such as are known to occur in red tide areas (Galtsoff, 1949) or as a result of unbalanced overfertilization such as is induced by organic wastes from duck farms in Great South Bay, N.Y. Turbidity may be determined practically by use of a Secchi disc. Turbidity may be determined more accurately by using the techniques described in Standard Methods for the Examination of Water and Wastewater, 12th edition (1965). Any turbidity of less than 1 m (by Secchi disc) or in corresponding Jackson units should be regarded with suspicion and the nature of suspended material as well as the composition of plankton determined.

Color

The color of sea water, expressed as dominant wave length in millimicrons (mμ) covers the range from violet (400 to 465 mμ) to red-purple (530 to 700 mμ). Spectrophotometric methods, as described in Standard Methods for the Examination of Water and Wastewater, 12th edition (1965), should be used if careful study is required, particularly for determining the exact color of water contaminated with industrial wastes.

Monitoring the color changes of sea water yields information on the extent of intrusion of fresh water into the sea, the intensity and extent of silting, the location and extent of plankton blooms, the extent and distribution of pollution from industrial waste effluents, and the presence and probable thickness of oil film.

In brackish waters, the blue hue of the open sea is replaced by a greenish or yellowish color. Silting areas are recognizable by brown or yellowish discoloration. Red-brownish color is typical of the red tide caused by *Gymnodinium* and other species of dinoflagellates. Some of these are toxic to fishes and benthic invertebrates (Galtsoff, 1948, 1949). Mass production of forms such as the blue-green alga *Trichodesmium* gives the surface of the sea an appearance of "green meadow" as described for the Azov Sea by Knipowich (Galtsoff, 1949). Swarming of *Phaeocystis poucheti*, *P. globosa*, and *Rhizosolenia* have been reported to extend over hundreds of square miles of the open sea causing a distinct brownish discoloration.

Systematic studies have not been made yet to determine the optical characteristics of discolored sea water. It is reasonable to expect that such an investigation would be valuable in explaining the cause of discoloration and, in certain instances, may indicate the presence and nature of pollution. Light components specific for the contaminant entering sea water may be detected by the use of a spectrophotometer or with the recording SPOT spectroradiometer recently developed by Alfred C. Konrad of the Massachusetts Institute of Technology. This type of instrument is being used at present at the Woods Hole Oceanographic Institution and is proving very useful. Spectroradiometer observations can be made either from an airplane or from shipboard.

Recommendation: No effluent which may cause changes in turbidity or color should be added to, or discharged into, inshore or coastal waters unless it has been shown that it will not be deleterious to aquatic biota.

Settleable and floating substances

Settleable solids entering coastal waters include various products of forest industries such as sawdust, bark chips, wood fibers, sewage solids, and many industrial wastes. The old practice of dumping sawdust into tidal rivers was discontinued long ago, but its effect is still visible in the rivers of Maine. For instance, an area of the bottom of the Damariscotta River was still covered with a loose layer of sawdust about 2 to 3 feet deep in 1940, although operation of the lumber mills responsible for this deposition had ceased more than 50 years previous. The Damariscotta kitchen-midden on the banks of the river contains a huge accumulation of river oyster shells and some artifacts left here by the Indians who lived there for several centuries of pre-Colonial times. The habitat was so completely changed by pollution that at present there is hardly any benthic organism found on this formerly productive bottom (Galtsoff and Chipman, unpublished report).

Decay-resisting organic matter from wood fibers and waterlogged bark and chips constitutes, in places, a serious handicap to aquatic life. Settleable materials from mining operations and gravel and sand washing make the bottom unsuitable for aquatic life in the affected areas of the receiving bodies of water. Silting may be so heavy that the sediment brought in may completely fill the bay. One can see this in the eastern branch of Matagorda Bay, Tex., an area that has been completely obliterated within the last 25 years by the Colorado River.

Dredging of bays and tidal rivers for improvement of navigation occasionally presents serious problems. Benthic communities in the area near dredging operations may be destroyed or damaged by spoil deposition, increase in water turbidity, release of toxic substances accumulated in the mud of the polluted areas, and by changing the pattern of currents in the dredged area.

Careful studies of the effects of dredging on oyster-producing bottoms of the Santee River, S.C., were made in 1936 by G. Robert Lunz, Jr. (unpublished report), for the U.S. Corps of Engineers. No deleterious effect on oyster-producing bottoms was found. An examination made by the Bureau of Fisheries Laboratory at Woods Hole of dredging operations to deepen and enlarge the Cape Cod Canal disclosed that several productive oyster beds near the site of dredging were covered by 2 to 3 feet of sand and silt. The oysters were destroyed, but the grounds soon were re-populated by hard-shell clams and the productivity of the area restored.

Disposal of the huge quantities of garbage accumulated by large cities presents a special and difficult problem. The old practice of barging this waste out to sea and dumping it is highly objectionable. Incineration seems to be the answer. This creates, however, the problem of proper incineration of large quantities of materials without increasing air pollution over the metropolis. The city of Boston disposes of large amounts of accumulated garbage and trash by incineration and by dumping the ashes into the sea at a distance from shore. State and Federal authorities are engaged presently in a study of the chemical composition of ash and its possible effect on aquatic life in the sea. Preliminary analysis of an incinerated sample made by Ronald Eisler (personal communication) of the National Marine Quality Laboratory of the Federal Water Pollution Control Administration shows that aluminum, iron, and calcium were most abundant, followed by zinc, sodium, potassium, and lead. Other metals comprising more than 1 percent of the fraction soluble in 6N HCl include barium, chromium, and magnesium. It is evident that ash from this waste contains a fairly large percentage of heavy metals which may be accumulating in the bodies of fish and shellfish. The effect of ash on the behavior of fish is now being studied, but the results are not yet available.

Examples of industrial effluents containing materials that precipitate in sea water are the waste from titanium paint plants or the soap portion of the effluents from Kraft pulp mills. This fraction of the black liquor is precipitated from solution by salt, carried by the current of the receiving river, and eventually deposited on the bottom (Galtsoff, et al., 1947). Waste from several plants extracting titanium dioxide from ilmenite (ferrous titanate) produces serious pollution in the lower Patapsco River area near Baltimore. Because of the restricted circulation of water in the upper Chesapeake Bay, the effect is quite pronounced. Ferric hydroxide flocculation in the Patapsco River has been found detrimental to plankton. Diatoms were destroyed by flocculation and removed from plankton by settling with the iron particles. Considerable amounts of iron accumulated on the bottom and iron precipitate was found coating the gills of minnows, silverside, and white perch (Olson, et al., 1941).

Recommendation: Water quality requirements for specifying the permissible limits of settleable solids and floating materials cannot be expressed quantitatively at present. Since it is known that even minor deposits may reduce productivity and alter the benthic environment, it is recommended that no materials containing settleable solids or substances that may precipitate out in quantities that adversely affect the biota should be introduced into estuarine or coastal waters. It is especially urgent that areas serving as habitat or nursery grounds for commercially important species (scallops, lobsters, oysters, clams, crabs, shrimp, halibut, flounders, demersal fish eggs and larvae, and other bottom forms) be protected from any infringement on natural conditions.

Tainting Substances

Substances found in industrial wastes are frequently responsible for objectionable or offensive tastes, odors, and colors of fish and shellfish. Even slight amounts of oil or petroleum products in bays and estuaries will impart an oil or kerosene flavor to mullet, mackerel, and other fishes and also to oysters, clams, and mussels making them unmarketable. Oysters collected in Louisiana waters polluted by crude oil retained a distinct flavor and odor associated with this type of pollution for several weeks after the escape of crude oil from wells and leaky barges had been stopped (Galtsoff, et al., 1935).

Anaerobic conditions associated with the deposit of sewage sludge on the bottom are accompanied by the production of hydrogen sulfide, a substance that causes black discoloration of bivalve shells and imparts an offensive flavor and odor to their flesh. In the waters receiving black liquor from Kraft pulp mills in the York River, Va., the gills and mantles of oysters developed a gray color. This condition also is found in oysters grown in waters receiving domestic sewage (Galtsoff, et al., 1957).

Contamination of water with copper results in the accumulation and storage of this metal far above its normal content in the tissues. The copper content of oyster flesh from uncontaminated waters off Cape Cod varied from 0.170 to 0.214 mg copper per oyster or from 8.21 to 13.77 mg per 100 g dry weight. In green colored oysters collected from adjacent areas only slightly contaminated with copper salts, the copper content in the flesh ranged from 1.27 to 2.46 mg per oyster or from 121.71 to 271 mg per 100 g dry weight (Galtsoff and Whipple, 1931; Galtsoff, 1964).

In a current study conducted at the Northeast Marine Health Sciences Laboratory, at Narragansett, R.I., Dr. B. H. Pringle (unpublished data) found that the average copper content of oysters collected from unpolluted areas along the east shore ranged from 20 to 80 mg/l, wet weight; oysters from areas known to be polluted contained from 124.5 to 392.0 mg/l wet weight. The copper content of sea water ranged between 0.0038 to 0.005 mg/l in areas not known to be polluted. In certain polluted places, concentrations as high as 0.019 mg/l were recorded.

Other metals are easily absorbed, stored, and concentrated by oysters in great excess of their concentration in sea water. Experimentally, it has been shown that iron and iodine can be absorbed within a relatively short time by oysters from water to which these metals have been added in excess. The flavor of so-called superiodized oysters produced before World War II in Arcachon, France, was pronounced because the iodine content of flesh was many times higher than that in untreated oysters (Galtsoff, 1964). The color of the oysters was not affected.

Green color of the gills of the European oyster in France and in the American oyster occasionally found in North Carolina and Chesapeake Bay is due to absorption of the blue-green pigment of the diatom, *Navicula*, present in large numbers on oyster grounds. The color is not associated with the increased copper content of flesh (Ranson, 1927).

Recommendation: To prevent the tainting of fish and other marine organisms, substances that produce tastes and off-flavors should not be present in concentrations above those shown to be acceptable by means of bioassays and taste panels. Experience has shown that test organisms should be exposed to the materials under test for 2 weeks at selected concentrations to determine the maximum concentration that does not produce noticeable off-flavors as determined by organoleptic tests. (Cooking should be done by baking the material wrapped in aluminum foil.)

Plant Nutrients and Nuisance Organisms

Plant nutrients and nuisance organisms are interrelated in many ways. There also are many other factors in the environment, such as temperature and salinity, that are closely correlated and, in many instances, seem to be contributing factors to nuisance organisms.

Man, through altering the hydrography of his environment by building dams and diverting waterflows from their natural courses, has produced conditions in many areas that have caused nuisance growths and brought about an imbalance of natural conditions. He also has enriched surface waters and created imbalances in dissolved materials and organisms through careless land management and by allowing the introduction of nutrients from sewers, food processing industries, fertilizer plants, feed lots, and farms. As a result, natural communities of aquatic life are altered and the functioning of these ecosystems often is changed severely or destroyed.

To maintain natural distribution, abundance, and interrelations of the aquatic biota, and to control unwanted growths, it is necessary to determine and maintain levels of dissolved materials required for this balance. This is an extremely difficult task, however, because there are a great many interrelated factors that contribute to the development of excessive populations of a species. Although a considerable amount of work has been done on the nutrition of aquatic organisms, most of this work has been done on a very few different species. Very little research has been done to determine what interaction of factors causes a shift in diversity or in the kinds of species that compose a community. For these reasons it is impossible to set any definite requirements. At this time the only meaningful thing that can be done is to develop guidelines.

Plant Nutrients: The increase of nutrients in the sea is accelerated by deposition of material derived from the land as sediments from the rivers, by settling and filling caused by water movements produced by tide or wind, and by biological activity. To date, no serious problems resulting from abnormal enrichment of nutrients have been identified in the open sea except perhaps locally around outfalls that extend several miles out to sea. With the increased disposal of wastes in the sea, this potential problem should be carefully watched.

Estuaries and tidal embayments have long been recognized as some of our most valuable and productive resources. They are the most ephemeral of the natural marine habitats and consequently most easily affected by man's activities. They serve as sinks for most of the organic and inorganic materials resulting from land erosion. Because of the lack of scouring and the nature of the sediments that occur in some areas, anaerobic conditions often develop in the beds of estuaries and bays. Increases in the deposition of suspended solids intensifies this condition. An excellent discussion of the role of sediments in an estuary is given by Carriker (1967).

Many industries and municipalities discharge nutrient-rich wastes into estuaries. Because of the nature of the estuary, these are recycled and accumulated over a period of time. Because of this recycling, effluents with low concentrations of nutrients may, in time, produce serious problems. The complete flushing of the estuary often takes many years. With controlled water discharges, this problem may become more severe.

Plant nutrients consist of many types of chemicals. For example, we have the chemicals commonly recognized as being important in plant nutrition such as nitrate, phosphate, sulfate, carbonate, calcium, magnesium, sodium, and potassium. There are also the so-called "trace elements" which are equally important but are required in small amounts such as iron, manganese, molybdenum, cobalt, zinc, etc. More recently the importance of organic compounds in plant nutrition has been recognized. These include vitamins, such as vitamin B_{12}, organic forms of nitrogen, such as urea, various amino acids, and amides, and the simple sugars, such as glucose.

The role of dissolved organic compounds in the nutrition of plants as well as animals appears to be important. Darnell (1967) refers to the aquatic medium as a "vegetable soup" to indicate its richness in dissolved organic materials. The work of Ryther (1954) points out that the organic forms of nitrogen are best utilized by the less desirable species (*Nannachloris atomus* and *Stichoccus sp.*). *Nitzschia*, a desirable diatom, often grows poorly in their presence. This is no doubt a major reason why sewage effluents often bring about the development of undesirable species.

If the increased nutrients in a system are well balanced, many species will have larger populations, the predator pressure will increase, and the productivity of the whole ecosystem will increase. If, however, the increased nutrients are of undesirable composition for most forms of aquatic life, or not in the correct ratio, excessive blooms of species with low predator pressures may develop. Examples of these are certain blue-green algae. Of course, environmental factors other than nutrients are important in the development of blooms. Any one important factor, such as temperature, light, or water mass stability, if limiting, may prevent blooms even though other conditions are suitable for their development. As a result, blooms sometimes do not develop even though most of the conditions are favorable.

Nuisance Organisms: Nuisance organisms in the marine environment are usually defined as those organisms which interfere with the use that man wishes to make of a particular water. Some examples are abnormally abundant growths of organisms that make bathing beaches unattractive, produce unpleasant odors, foul the bottoms of boats, spoil the esthetic appearance of water and the coastline, clog fishing nets, interfere with the flow of water within intake and effluent pipes, and interfere with navigation. This category of nuisance organisms should also include those organisms that interfere with the growth and reproduction of organisms important to man. For example, excessive populations of boring sponge or oyster drills, rooted and floating aquatics can interfere with the movement and reproduction of fish; bacteria and red tide organisms such as *Gymnodinium* and *Gonyaulax* may have toxic effects on other organisms, including man (Rounsefell and Nelson, 1966; Felsing, 1966).

The groups of organisms that may cause nuisances or become severe pests include algae (including red tide organisms), coelenterates, sponges, mollusks, such as oyster drills and mussels, and crustacea. These organisms are commonly encountered in the natural marine environment. Organisms may become nuisances because of excessive growth and changes in distribution patterns and predator-prey relationships. The main causative factors are excessive and, often, imbalanced nutrients, considerable changes in the natural regimes of temperature, turbidity, and salinity, and changes in current patterns.

In some instances, nuisance growths seem to be directly related to the nutrients that are available. In other situations, nuisance growths may not be directly affected by artificial enrichment, so far as we know, and seem to be more strongly affected by changes in the temperature, salinity, or turbidity. Included here are various fouling organisms: barnacles, mussels and other mollusks, polyzoa tube worms, marine borers, and pests to useful marine products (oyster drills, boring sponges, crabs, parasitic fungi, and protozoans), and swarms of jellyfish, which make bathing in some coastal waters hazardous during certain seasons.

The effect of increased nutrients may be an increase in the populations of certain species already present in the environment and a decrease of species that are not tolerant of such nutrients. Examples of such conditions are the increase of *Enteromorpha* and sea lettuce, *Ulva lactuca*, in the zone of mineralization of sewage which occurs in some areas of the lower Potomac. In areas of higher salinity, abundant growths of *Ascophyllum* often occur in waters containing mineralized effluents from sewage treatment plants. In Biscayne Bay, Fla., the following organisms became abundant under such conditions: the flowering plants, *Halophila baillonis* and *Diplanthera wrightii;* and the echinoderm, *Amphioplus abditus*. Under heavy organic enrichment, the algae, *Gracilaria blodgettii* and *Agardhiella tenera*, the worm, *Diopatra cupera*, and the amphipods, *Erichthonius brasiliensis* and *Corophium acherusicum*, became very common (McNulty, 1955).

In other cases, an imbalanced organic enrichment together with changes in temperature and salinity brings about an almost complete change in the species composing an aquatic community plus excessive growths of some species. An excellent example of this type has been described by Ryther (1954) in his studies of Moriches Bay and Great South Bay, Long Island. In this area, duck farm wastes enrich the bay waters with organic compounds that produce a low nitrogen-to-phosphorus ratio. At the times of the largest algal blooms, low salinities and high temperatures exist in the area. As a result, desirable marine diatom species of *Nitzschia* which prefer cool water (5 to 25 C), nitrates, and nitrites as a source of nitrogen, and are not benefited by a low N/P ratio (5:1) were replaced by *Nannochloris atomus* and *Stichococcus sp*. These species can grow well in nitrates, nitrites, ammonia, urea, uric acid, and cystine, and prefer a N/P ratio of 5:1. As Ketchum (1967) points out, these weed species are undesirable food sources and the natural productivity of the estuary is destroyed. Ketchum also points out that the greatest amount of plankton does not always occur in the waters of greatest enrichment. This is because the development of a maximum standing crop of phytoplankton is also governed by the concentration of predators, stability of the water column, transparency of the water, etc.

Nutrient imbalance may affect the ratio of inorganic phosphate to total phosphorus, here defined as the sum of inorganic, organic, and particulate phosphorus. It is known from the work of Pomeroy (1960) and others that inorganic phosphorus is rapidly taken up by actively growing plants. At the same time, inorganic phosphorus is regenerated as a result of bacterial degradation and excretion by animals. The net effect over the short run is to produce a steady state between the various fractions of phosphorus in the environment. There should be some ratio of inorganic to total phosphorus in the euphotic zone that would be characteristic of a balanced nutrient regime and this ratio should be lower than the same ratio for the imbalanced system in which inorganic phosphorus can accumulate.

Data from Moriches Bay and Great South Bay on Long Island, Charlestown Pond, R.I., the North Atlantic, and the North Pacific have been examined. In the obviously polluted portion of Moriches Bay, the inorganic total phosphate ratio generally exceeds 0.6, while the Charlestown Pond, an uncontaminated estuary of similar characteristics to Moriches Bay, this ratio was less than 0.4. In the open ocean at high latitudes and in the winter when phytoplankton density is low, the fraction of inorganic phosphorus may increase to 0.65 or thereabout.

Recommendation: The ecological factors most often associated with nuisance growths are changes in the natural temperature and salinity cycles and increases in nutrients. The change in any of these factors may directly or indirectly affect the response of the organisms to other factors. Increase or decrease in current and, indirectly, its effect on available nutritional materials have also been found to be important.

To maintain a balance among nutrients and a balanced biota most conducive to the production of a desired crop, it is recommended that:

(1) No changes should be made in the basin geometry, current structure, salinity, or temperature of the estuary without first studying the effects on aquatic life. For example, these studies should be made before dams are erected, water diversion projects are constructed, or dredge and fill operations carried out.

(2) The artificial enrichment of the marine environment from all sources should not cause any major quantitative or qualitative alteration in the flora. Production of persistant blooms of phytoplankton, whether toxic or not, dense growths of attached algae or higher aquatics or any other sort of nuisance that can be directly attributed to nutrient excess or imbalance should be avoided. Because these nutrients often are derived largely from drainage from land, special attention should be given to correct land management in a river basin and on the shores of a bay to prevent erosion.

(3) The naturally occurring atomic ratio of NO_3-N to PO_4-P in a body of water should be maintained. Similarly, the ratio of inorganic phosphorus (orthophosphate) to total phosphorus (the sum of inorganic phosphorus, dissolved organic phosphorus, and particulate phosphorus) should be maintained as it occurs naturally. Imbalances have been shown to bring about a change in the natural diversity of the desirable organisms and to reduce productivity.

Toxic Substances

Relatively few of the many substances recognized as potential toxic pollutants of the marine environment have been studied sufficiently to enable us to define their maximum allowable concentrations. Specific pollutants and classes of pollutants are discussed in terms of current knowledge. In some cases, data are adequate to set definite criteria, while in others, criteria are educated guesses at best and can serve only as temporary guidelines.

Lethal concentrations of some persistent substances as determined by acute toxicity tests are so low that we are not justified in allowing their deliberate introduction into the natural environment. On the other hand, a few waste products appear to offer little threat to the marine environment because of their rapid degradation and dispersal.

Our concern is not primarily with what polluting substances are present, but whether or not they are present in sufficiently large amounts to cause deleterious effects on the biota and the environment. Many naturally occurring substances, including clean fresh water, would be toxic if discharged into the estuarine and coastal marine environment in sufficiently large amounts.

Determination of the toxicity of known and unknown effluents, either simple or complex mixtures, can best be made by determining the reactions of endemic fauna exposed to them at levels that might be expected in receiving waters. Chemical assays may determine the presence of such pollutants at levels as low as nanograms per liter, but biological systems may be affected by even smaller amounts. Many animals have the ability to accumulate toxic residues of substances present in the environment in only trace amounts until body residues are large enough to cause damage when released internally through normal metabolic processes. Animals differ in their sensitivity to the same toxicant and it is essential that toxicity data be related, in the final analysis, to animals of economic importance.

A fundamental concept in attacking the pollution problem is the assumption that effluents containing foreign materials are harmful and not permissible until laboratory tests have shown the reverse to be true. It is the obligation of the agency producing the effluent to demonstrate that it is harmless rather than require pollution abatement agencies to demonstrate that the effluent is causing damage.

Specific methods are suggested here for the determination of the toxicity of proposed effluents. While certain procedures are desirable, they are not always reasonable and certain permissible alternatives are also given.

Basic Bioassay Test: The basic bioassay test shall consist of a 96-hour exposure of an appropriate organism, in numbers adequate to assure statistical validity, to an array of concentrations of the substance, or mixture of substances, that will reveal the level of pollution that will cause (1) irreversible damage to 50 percent of the test organisms, and (2) the maximum concentration causing no apparent effect on the test organisms in 96 hours. Tests should be conducted, when possible, in a "flow-through" system so that the organisms are exposed continuously to a fresh solution of the test material appropriately diluted with water of the same quality as that at the site of the proposed discharge. Adequate safeguards should be taken to insure that the test will be conducted under the least favorable environmental conditions that are allowable in the natural environment. Tests should be conducted at water temperatures typical of the mean of maximum daily temperatures during critical periods at the proposed effluent discharge site.

Test organisms should be selected either on the basis of their economic importance in the area receiving the discharge and their sensitivity or on the basis of their importance in the food web of economically important animals. In the event that organisms meeting these criteria are not suitable or available for the confined conditions of the tests, substitute forms endemic to the area may be utilized. Appropriate tests must be undertaken to demonstrate the relative sensitivity of economically important species and substitute species to the test material so that meaningful interpretations of the data can be made.

Application Factor: It is recognized that the most obviously deleterious effect of toxic substances is increased mortality. More subtle changes such as reduced growth, lowered fecundity, altered physiology, and induced abnormal behavior patterns may have more disastrous effects on the continued existence of the species. Evaluations of such sublethal effects generally will provide more meaningful guidelines.

It is recognized that there should be an application factor for each waste or material and that these factors may vary widely for these different wastes and materials. The concept and use of application factors is defined and discussed at length in the toxicity portion of the section on water quality requirements for fresh water organisms. Due to a lack of knowledge of application factors for specific wastes and materials, a single application factor to be applied to all wastes is being suggested at this time. This application factor may require a lower concentration than is necessary in some instances, particularly for those materials that are subject to biological degradation, but it is known that it is not restrictive enough for some materials. Ideally, the determination of application factors should be the result of studies for the determination of safe levels of potential toxicants under long-term or continuous exposure. The application factor is the concentration of a material or waste that is not harmful, divided by the 96-hour TL_m value for that material. A few application factors have been so determined at the Bureau of Commercial Fisheries Laboratory at Gulf Breeze, Fla. (unpublished data). In the future, as application factors are determined for specific substances, they will replace the recommendation for the generalized application factor for these particular materials or wastes. It is clearly understood that as additional data become available recommendations on water quality requirements will be changed so that they conform with the new knowledge.

Biological Magnification: Biological magnification is an additional chronic effect of toxic pollutants (such as heavy metals, pesticides, radionuclides, bacteria, and viruses) which must be recognized and examined before clearance can be given for the disposal of a waste product into natural waters. Many animals, and especially shellfish such as the oyster, have the ability to remove from the environment and store in their tissues substances present at nontoxic levels in the surrounding water. This process may continue in the oyster or fish, for example, until the body burden of the toxicant reaches such levels that the animal's death would result if the pollutant were released into the bloodstream by physiological activity. This may occur, as in the case of chlorinated hydrocarbon pesticides (such as DDT and endrin) stored in fat depots, when the animals food supply is restricted and the body fat is mobilized. The appearance of the toxicant in the bloodstream causes the death of the animal. Equally disastrous is the mobilization of body fat to form sex products which may contain sufficiently high levels of the pollutant so that normal development of the young is impossible.

The biological magnification and storage of toxic residues of polluting substances and microorganisms may have another serious after effect. Herbivorous and carnivorous fish at lower trophic stages may gradually build up DDT residues of 15 to 20 mg/l without apparent ill effect. Carnivorous fish, mammals, and birds preying on these contaminated fish may be killed immediately or suffer irreparable damage because of the pesticide residue or infectious agent.

In the final analysis, laboratory tests alone are not sufficient to assess completely the toxic effects of a substance. These data must be interpreted in combination with field observations. Criteria established under the artificial conditions of laboratory tests will probably require adjustment in the light of later and more prolonged field observations.

Recommendation: In the absence of toxicity data other than the 96-hour TL_m, an arbitrary application factor of $\frac{1}{100}$ of this amount shall be used as the criterion of permissible levels.

Additional chronic exposure tests will be conducted within a reasonable period to demonstrate that the estimated maximum safe levels as indicated by the

96-hour TL$_m$ and the application factor do not, in fact, cause decreases in productivity of the test species during its life history.

Monitoring the Marine Environment: The chief problem in monitoring the marine habitat for pollution lies in the fact that the discharge of toxic materials may be intermittent. This is not necessarily true, but it means that water samples collected periodically reflect only the conditions at the time they were collected. Significantly higher or lower levels of pollution may have existed between sample collections. A second major factor for consideration is that trace amounts of pollutants or effluent mixtures toxic to the biota may not be readily susceptible to chemical analysis. For these reasons, the analysis of resident biota for abnormal changes offers a better tool for interpreting environmental fluctuations.

Mollusks are being collected for analysis at monthly intervals in estuaries on both the Atlantic and Pacific coasts (Butler, 1966 a, b). Analysis of resident populations by electron capture, gas chromatographic techniques reveal changes in residues of 11 of the more common organochloride pesticides which oysters, mussels, and some species of clams readily store. These methods are useful for rapid surveys of recent pollution. By appropriate spacing of samples in time and location, it has been possible to pinpoint sources of pollution.

It is suggested that a monitoring system of this type, appropriately expanded to include fish and plankton, would quickly identify areas where pollution problems exist. Suitable analytical techniques are available to make these samples equally useful for the identification of pollution by heavy metals and other toxic substances.

Monitoring for the presence of organophosphorous materials is feasible, but less specific for individual toxic compounds. This group of pesticides exerts its toxic effect on living systems by inhibiting the enzyme acetylcholinesterase, which is essential to conduction in nerve fibers. The nervous tissue of fish and some invertebrates, appropriately analyzed, reveals whether the organism has been exposed to organophosphorous materials within the past 2 to 4 weeks (Holland, et al., 1967). Identification of such changes can be made before toxicant levels are high enough to cause serious mortalities.

A particularly efficient nonspecific method for monitoring changes in the estuarine habitat is based on the periodic collection of sedentary animals and plants which have attached themselves to artificial cultch plates. Squares of asbestos cement boards placed in strategic locations will be utilized by resident biota as a habitat. At 30-day or shorter intervals these plates can be changed, the organisms enumerated, volumetrically measured or chemically assayed, and an index of their relative abundance obtained (Butler, 1954).

Such plates have been maintained for nearly 20 years at one laboratory in Florida (Butler, 1965), and they supply detailed information on the relative productivity of the environment in relation to hydrologic changes. They will be equally useful as monitors of newly introduced pollutants in this area. The monitoring method of choice—and there are others besides the ones suggested—will depend on the specific environment and the animals of particular interest. No one method will be adequate and a combination of methods should provide the most information in the shortest time period.

Pesticides: Pesticides may be described as natural and synthetic materials used to control unwanted or noxious animals and plants. They exert their effect as contact or systemic poisons, as repellents, or in some cases as attractants. It is convenient to classify them according to their major usage such as fungicides, herbicides, insecticides, fumigants, and rodenticides. Although data are not available as to the total amount of pesticides used in the United States, total production figures (including exports) show that more than 875 million pounds were produced in 1965. This represents an increase of approximately 10 percent over 1964, and more than a fivefold increase in the past two decades. In recent years, the use of herbicides has increased relatively more rapidly than that of other pesticides. In 1964, more than 100 million acres of the continental United States were treated with some kind of pesticide. The trend in pesticide production is towards the manufacture of more granular formulations. This physical adsorption of the pesticide on clay particles makes possible better control during application and should result in less dissipation of the chemical into atmosphere and into nontarget areas.

Despite better control of pesticide applications, their dispersal in drainage systems and possible eventual accumulation in estuaries makes our coastal fisheries especially vulnerable to their toxic effects. Estuarine oyster populations, juvenile shrimp, crab, and menhaden, for example, all occupy the habitat where fresh and salt water mix and where deposition of river silt with its load of adsorbed pollutants takes place. Laboratory tests show that these economically important animals are especially sensitive to the toxic effects of low levels of pesticides. Oysters, for example, will exist in the presence of DDT at levels as high as 0.1 mg/l in the environment. But at levels 1,000 times less (0.1 μg/l), oyster growth or production would be only 20 percent of normal, shrimp populations would suffer a 20-percent mortality, and menhaden would suffer a disastrous mortality. Some insecticides are toxic enough to kill 50 percent or more of shrimp populations after 48 hours exposure to concentrations of only 30 to 50 nanograms per liter of the compound.

Pesticides may be classified by their chemical affinities and a large number of economically important insecticides are chlorinated hydrocarbon compounds. These include the well-known DDT and aldrin-toxaphene group. Typically, these are persistent compounds, but they may be degraded by living systems into less toxic metabolites. As residues in soil and marine sediments, they may persist unchanged for many years and consequently present a continuing threat to animal communities. As a general rule, the acute toxicity of this group of pesticides increases with the level of metabolic activity so that their presence may cause two or three times more damage in summer than in winter months.

The organophosphorous pesticides are also primarily insecticides. Typically, they hydrolyze or break down into less toxic products much more readily than the organochloride compounds. Practically all persist for less than a year, while some last only a few days in the environment. Most of them are degraded rather quickly in warm water and consequently are more hazardous to aquatic animals at winter rather than summer temperatures. They exhibit a wide range of toxicity, both more and less damaging to marine fauna than the organochlorides. They are usually preferable as control agents because of their relatively short life.

Other major chemical categories including the carbamates, arsenicals, and 2,4-D and 2,4,5-T compounds are generally, but not necessarily, less toxic to marine biota.

Pesticides registered for uses which might permit their dispersal into the marine environment must be evaluated for their toxic effect on oysters, fish, and shrimp. Consequently, there is a considerable amount of information on the 48 or 96-hour TL_m values of these compounds. Unfortunately, information is still lacking on their long-term effects at sublethal levels on the productivity of economically important marine species.

The extreme sensitivity of marine crustaceans, such as crabs, lobsters, and shrimp, to the array of insecticides is to be expected because of their phylogenetic relationship with terrestrial arthropods. In general, shrimp are also much more sensitive than fish or oysters to the other pesticides. This fact and their economic importance make shrimp a valuable yardstick for establishing safe levels of pesticides that might be expected as toxicants in the marine environment.

A much broader spectrum of pesticide pollutants can be anticipated in the fresh water (salinity <0.5 ‰) zones of tidal estuaries. Fresh water criteria listed in another section will apply under these circumstances.

Recommendation: The pesticides are grouped according to their relative toxicity to shrimp, one of the most sensitive groups of marine organisms. Criteria are based on the best estimates in the light of present knowledge and it is expected that acceptable levels of toxic materials may be changed as the result of future research.

Pesticide group A.—The following chemicals are acutely toxic at concentrations of 5 μg/1 and less. On the assumption that $^1/_{100}$ of this level represents a reasonable application factor, it is recommended that environmental levels of these substances not be permitted to rise above 50 nanograms/1. This criterion is so low that these pesticides could not be applied directly in or near the marine habitat without danger of causing damage. The 48-hour TL_m is listed for each chemical in parts per billion (μg/l).

Organochloride pesticides

Aldrin	0.04	DDT	0.6
BHC	2.0	Dieldrin	0.3
Chlordane	2.0	Endosulfan	0.2
Endrin	0.2	Methoxychlor	4.0
Heptachlor	0.2	Perthane	3.0
Lindane	0.2	TDE	3.0
		Toxaphene	3.0

Organophosphorous pesticides

Coumaphos	2.0	Naled	3.0
Dursban	3.0	Parathion	1.0
Fenthion	0.03	Ronnel	5.0

Pesticide group B.—The following types of pesticide compounds generally are not acutely toxic at concentrations of 1 mg/1 or less. It is recommended that an application factor of $^1/_{100}$ be used and, in the absence of acute toxicity data that environmental levels of not more than 10 μg/1 be permitted.

Arsenicals
Botanicals
Carbamates
2,4-D compounds
2,4,5-T compounds.
Phthalic acid compounds.
Triazine compounds.
Substituted urea compounds.

Other pesticides.—Acute toxicity data are available for approximately one hundred technical grade pesticides in general use not listed in the above groups. These chemicals either are not likely to reach the marine environment, or, if used as directed by the registered label, probably would not occur at levels toxic to marine biota. It is presumed that criteria established for these chemicals in fresh water will protect adequately the marine habitat. It should be emphasized that no unlisted chemical should be discharged into the estuary or coastal water without preliminary bioassay tests and the establishment of an adequate application factor.

Heavy Metals: Heavy metal salts in solution may constitute a very serious form of pollution because they are stable compounds, not readily removed by oxidation, precipitation, or any other natural process. A characteristic feature of heavy metal pollution is its persistence in time as well as in space for years after the pollutional operations have ceased.

The number of substances that may be described as "poisonous" is very large and they vary enormously in the degree of their effect. For man and other air-breathing animals, the threshold dose of a toxic material generally means the maximum quantity that can be taken without causing death. For aquatic animals living in a water environment containing a toxic substance, the situation is somewhat different. Instead of receiving an absolute quantity at one time, they are being continually exposed to a given concentration of the toxic material. This is similar to a man regularly drinking water containing lead or breathing air containing a noxious gas or vapor. It is not surprising, therefore, that the student of pollution problems turns his attention toward the concentration of the poison he is investigating and the manner in which the effect is related to this, rather than to the absolute amount required to harm or kill. Animals have the ability to eliminate poisons at least to some degree or even to destroy them. Their ability to do this at a rate permitting survival depends on the concentration of the toxic material to which they are exposed.

One of the characteristics of living cells is their ability to take up elements from a solution against a concentration gradient. This is perhaps most obvious for marine organisms, especially for autotrophic algae which obtain all their nutrients directly from seawater. The ability of marine organisms to concentrate elements above that level found in their environment has been recognized for some time. The following points should be noted in relation to their concentrating ability.

(1) All elements are concentrated to a degree with the exception of chlorine, which is rejected, and sodium, which is weakly rejected. The concentration factors are of the order of one for bromine, fluorine, magnesium, sodium, and sulfur, and higher for all other elements.

(2) Among cations (including metallic elements such as iron, which may exist as colloids in the sea), the order of affinity for living matter is, generally: tetravalent and trivalent elements>divalent transition elements>divalent group II-A metals>univalent group I metals. The tetravalent and trivalent subgroup have rather different affinities for plankton and brown algae.

plankton: Fe>Al>Ti>Cr, Si>Ga
brown algae: Fe>La>Cr>Ga>Li>Al>Si

Similar differences are found between these organisms in their affinities for the divalent transition metals.

plankton: Zn>Pb>Cu>Mn>Co>Ni>Cd
brown algae: Pb>Mn>Zn>Cu, Cd>Co>Ni

Of interest is the affinity of both organisms for lead, which has no known biological function.

It is clear that the heavier elements in these groups tend to be more readily taken up than the lighter ones, which may be connected with their greater, ease of polarization.

(3) The order of affinity of living matter for anions is:

nitrate>trivalent anions>divalent anions>univalent anions

It is probable that most polyvalent metallic elements are more or less chelated by organic matter.

The main features of the uptake of ions by cells can be accounted for by assuming that another process operates apart from simple diffusion. This process is called active uptake and is closely linked with metabolic activities within the cell. The metabolic processes provide the energy necessary for the uptake against a concentration gradient. Active uptake has a larger temperature coefficient than does uptake by diffusion. In long-term experiments, the effect of temperature is probably complicated by increased rates of growth, cell division, and so on. Active uptake requires oxygen and occurs only in cells which are respiring freely. Substances which inhibit respiration also inhibit uptake of ions. The rate of uptake of ions may be limited either by the rate of exchange at the cell membrane or by bulk phase diffusion inside the cell. The former is usually limiting for ions present at low external concentration and the latter for ions at high external concentrations. It has been suggested that bulk phase diffusion limits the rates of uptake of most cations. There appears to be at least two active transport systems in addition to the diffusion processes. A large number of theories have been advanced to explain active transport. One of the most popular is the carrier hypothesis. Accordingly, the ions are transported across membranes as chelates with metabolically produced organic molecules.

Uptake by invertebrate animals.—The most primitive animals, the unicellular protozoa, take up ions from solution by diffusion in the same ways as do algae. Many marine species have vacuoles and these are able to open at intervals and extrude fluid from the cell. The vacuole regulates the osmotic pressure of the cell and thus controls its volume.

Multicellular invertebrate animals can be divided into two groups as far as uptake is concerned: those with permeable integuments and those without. The majority of marine invertebrates (colenterates, annelids, mollusks, and echinoderms) have soft bodies with permeable integuments through which ions can diffuse freely. In this situation, the body fluid or blood is quite similar to sea water in composition. The gills of mollusks are coated with a layer of complex carbohydrate sulfates which may function as ion exchangers. The gills of marine Crustacea, which have hard impermeable carapaces, are fully permeable to water and salts.

Mode of toxic action.—An element is said to be toxic if it injures the growth or metabolism of an organism when supplied above a certain concentration. All elements are toxic at high concentrations and some are notorious poisons even at low concentrations. For example, the essential micronutrient, copper, which is a necessary constituent of all organisms, is highly toxic at quite small concentrations. The other essential micronutrients are also toxic when supplied in excess, though not all in such striking fashion. There is an optimum range of concentration, which is sometimes quite narrow, for the supply of each element to each organism.

When excessive amounts of an element are fed to an organism, they frequently cause death. The usual measure of the amount required to cause death is called the LD_{50}. This is the amount which, when fed to each individual in a population, kills half of the population. The LD_{50} is an imprecise measure unless it is qualified by specifying:

(1) The chemical state of the element.
(2) The means of feeding.
(3) The age or developmental stage of the organism.
(4) The time elapsed between feeding and death.

The most important mechanism of toxic action is thought to be the poisoning of enzyme systems. The more electronegative metals, notably copper, mercury, and silver, have a great affinity for amino, imino, and sulfhydryl groups which are doubtless reactive sites on many enzymes. These metals are readily chelated by organic molecules. We thus have discovered attempts to correlate metal toxicities with such factors as their electronegativities, the insolubility of their sulfides, or the order of stability of their chelated derivatives:

(1) Order of electronegativities of some divalent metals: Hg>Cu>Sn>Pb>Ni>Co>Cd>Fe>Zn>Mn>Mg>Ca>Sr>Ba
(2) Order of stability products of the sulfides: Hg>Cu>Pb>Cd>Co>Ni>Zn>Fe>Mn>Sn>Mg>Ca
(3) Order of stability of chelates: Hg>Cu>Ni>Pb>Co>Zn>Cd>Fe>Mn>Mg>Ca.

It appears likely that all the divalent transition metals, as well as the other electronegative metals, that form insoluble sulfides, such as Ag, Mo, Sb, Tl, and W, are poisons by virtue of their reactivity with proteins and especially with enzymes. In view of the large number of enzymes in living cells, the variations in toxicity indicated above are hardly surprising. Studies have shown that metals giving rise to similar toxic effects may be acting on quite unrelated enzymes and also many more atoms of metal are absorbed by an inactivated enzyme than are required to block the reactive sites. Other modes of toxic action are:

(1) Substances behaving as antimetabolites. This might be arsenate and chlorate occupying sites for phosphates and nitrates, respectively. (Fluoride, borate, bromate, permanganate, antimonate, selenate, tellurate, tungstate, and beryllium.)
(2) Substances forming stable precipitates or chelates with essential metabolites. (Al, Be, Sc, Tl, Y, Zr, reacting with phosphate, Ba with sulfate, or Fe with ATP.)
(3) Substances catalyzing the decomposition of essential metabolites. (La and other lanthanide cations decompose ATP.)
(4) Substances combining with the cell membrane and affecting its permeability. (Au, Cd, Cu, Hg, Pb, U.) These elements may affect transport of sodium, potassium, chlorine, or organic molecules across membranes or even rupture them.
(5) Substances replacing structurally or electrochemically important elements in the cell and then failing to function. (Li replacing Na, Cs replacing K, or Br replacing Cl.)

Metal-organic compounds may be either more toxic than the metal ion (ethyl mercuric chloride) or much less so (cupric ion and copper salicylaldoxime).

Silver.—Silver is present in seawater in a concentration of about 0.0003 mg/l. It is found in marine algae at concentrations up to 0.25 mg/l and in marine mammals in the range of 1 to 3 mg/l (Vinogradov, 1953). It is highly toxic to plants and mammals.

Arsenic.—Arsenic is found to a small extent in nature in the elemental form. It occurs mostly in the form of arsenites of true metals or as pyrites.

Its major commercial use is for pesticides (insects, weeds, fungi). Arsenic is cumulative in the tissues of many organisms and, therefore, it eventually exerts its effects even though the environmental level is low. It has been demonstrated to be a possible carcinogen in water.

Arsenic is found in seawater at a concentration of about 0.003 mg/l. It has been found in marine plants at concentrations up to 30 mg/l and is highest in the brown algae. It is found in marine animals in a range of 0.005 to 0.3 mg/l. It is accumulated by coelenterates, some mollusks, and crustaceans (Vinogradov, 1953). It is moderately toxic to plants and highly toxic to animals especially as AsH_3.

Arsenic trioxide, which also is exceedingly toxic, was studied in concentrations of 1.96 to 40 mg/l and found to be harmful to fish or other aquatic life. Work by the Washington Department of Fisheries (1944) on pink salmon has shown that at a level of 5.3 mg/l of As_2O_3 for 8 days was extremely harmful to this species. Ellis (1937), using the same compound on mussels at a level of 16 mg/l, found it to be quite lethal in 3 to 16 days. Surber and Meehan (1931) carried out an extensive study on the toxicity of As_2O_3 to many different fish food organisms. Their results indicated that important fish food organisms can tolerate an application rate of 2 mg/l of As_2O_3. The amount actually in the water is considerably less.

Cadmium.—The elemental form of cadmium is insoluble in water. It occurs largely as the sulfide which is often an impurity in zinc ores.

Cadmium is found in seawater at a level of less than 0.08 mg/l. Its level in marine plants is approximately 0.4 mg/l, while in marine animals a range of 0.15 to 3 mg/l has been found. It is lowest in the calcareous tissues and is accumulated within the viscera of the mollusk, *Pecten novazetlandicae* (Brooks and Rumsby, 1965). Cadmium is moderately toxic to all organisms and it is a cumulative poison in mammals.

Cadmium is used widely industrially to alloy with copper, lead, silver, aluminum, and nickel. It is also used in electroplating, ceramics, pigmentation, photography, and nuclear reactors. Cadmium salts sometimes are used as insecticides and antihelminthics. The chloride, nitrate, and sulfate of cadmium are highly soluble in water. The carbonate and hydroxide are insoluble, thus cadmium will be precipitated at high pH values.

Most quantitative data on the toxicity of cadmium are based on specific salts of the metal. Expressed as cadmium, these data indicate that the acute lethal level for fish varies from about 0.01 to about 10 mg/l depending on the test animal, the type of water, temperature, and time of exposure. Cadmium acts synergistically with other substances to increase toxicity. Concentrations of 0.03 mg/l in combination with 0.15 mg/l zinc causes mortality of salmon fry (Hublou, et al., 1954).

Pringle (in press), in a study of adult American Eastern oysters, *Crassostrea virginica*, found an 8-week TL_m value of 0.2 mg/l of $Cd^{++}[Cd(NO_3)_2]$ and a 15-week TL_m value of 0.1 mg/l.

The most obvious effect, in addition to lethality, was lack of shell growth. A similar study on the clam, *Mercenaria mercenaria*, indicated that a much longer period of exposure at the same concentration was required to kill half of the test organisms.

Chromium.—Chromium is found in seawater at a concentration of 0.00005 mg/l. Marine plants contain approximately 1.0 mg/l while marine animals contain chromium within a range of 0.2 to 1.0 mg/l. Chromium compounds may be present in wastes from many industrial processes or they may be discharged in chromium-treated cooling waters. The toxicity of chromium varies with the species, temperature, pH, its valence, and synergistic or antagonistic effects (especially with hardness). Most evidence points to the fact that under long-term exposure the hexavalent form is no more toxic towards fish than the trivalent form. Doudoroff and Katz (1953), studied the effect of $K_2Cr_2O_7$ on mummichaugs and found that they tolerated a 200 mg/l level in sea water for over a week.

The effects of hexavalent chromium on photosynthesis by the giant kelp, *Macrocystis pyrifera*, were as follows: at 1 mg/l chromium, photosynthesis was not diminished by 2 days contact. It was reduced 10 to 20 percent by 5 days contact and 20 to 30 percent after 7 to 9 days. The concentration of chromium required to cause a 50-percent inactivation of photosynthesis in 4 days was estimated at 5 mg/l (Clendenning and North, 1958, 1960; North and Clendenning, 1958, 1959).

Haydu (unpublished data) studied oyster mortalities and his results point out the long-term effects of low concentrations of chromium, molybdenum, and nickel. The levels of all three metals were in the range of 10 to 12 μg/l over a 2-year period. In addition, his data indicated that there were seasonal variations. The mortalities at these levels increased with an increase in temperature. Approximately 63 to 73 percent of the mortalities occurred in the period of May through July, perhaps due to increased physiological activity (increased feeding and higher pumping rates).

This study substantiates the available evidence indicating that as the environmental level of these metals increases, the ingestion-elimination balance is upset, causing accumulation to take place.

Raymont and Shields (1964), in studies with the small prawn, *Leander squilla,* found a threshold level of a little less than 5 mg/l Cr. Thus, at chromium concentrations ranging from 10 to 80 mg/l Cr, 100-percent mortality occurred in 1 week; at 5 mg/l Cr no deaths occurred in 1 week although a few animals died over the subsequent 21 days. Larger prawns of the same species appeared to be considerably more resistant to chromium poisoning. The threshold was about 10 mg/l Cr. Raymont and Shields in additional experiments on the toxicity of chromium to crustaceans (the shore crab, *Carcinus maenas*), indicated that chromium concentrations above 50 mg/l (Na_2CrO_4) were definitely toxic for a period of exposure of 12 days. At 60 mg/l Cr, 50-percent mortality occurred after 12 days. At 40 mg/l Cr, 9 percent died within 12 days, while at 20 mg/l, an 8-percent mortality was observed. In studies on the marine polychaete worm, *Nereis virens,* these same investigators working in the range of 2 to 10 mg/l Cr found that there was heavy mortality with all solutions in 2 to 3 weeks. The threshold of toxicity appears to be at about 1.0 mg/l Cr level.

Pringle (in press), in experiments using a well-controlled, flow-through system and chromium concentrations of 0.1 and 0.2 mg/l ($Na_2Cr_2O_7$), showed the average weekly mortality to be approximately 1 percent over a 20-week period. This was about the same as that for the sea water controls.

Copper.—Copper is found in seawater at a level of 0.003 mg/l. It is found in marine plants at about 11 mg/l, while marine animals are found to contain 4 to 50 mg/l. It is accumulated by some sponges and is essential for the respiratory pigment in the blood of certain annelids, crustacea, and mollusks. In excess, it is highly toxic to algae, seed plants, and to invertebrates and moderately toxic to mammals. Copper is not considered to be a cumulative systemic poison like lead or mercury.

The toxicity of copper to aquatic organisms varies significantly not only with the species but also with the physical and chemical characteristics of the water. Copper acts synergistically with zinc, cadmium, and mercury, yet there is a sparing action with calcium.

Barnacles and related marine fouling organisms were killed in 2 hours by 10 to 30 mg/l copper. Clarke (1947) showed that the mussel, *Mytilus edulis,* was killed in 12 hours by 0.55 mg/l. Lobsters transferred to tanks lined with copper after living in aluminum, stainless steel, and iron tanks for 2 months, died within 1 day. Copper is concentrated by plankton from surrounding water in ratios of 1,000 to 5,000 or more (Krumholz and Foster, 1957).

Concentrations of copper above 0.1 to 0.5 mg/l were found to be toxic to oysters by Galtsoff (1932). The 96-hour TL_m for oysters was estimated at 1.9 mg/l (Fujiya, 1960). Oysters cultured in waters containing 0.13 to 0.5 mg/l accumulated copper in their tissues and became unfit as a food substance. Pringle (in press) found the soft clam, *Mya arenaria,* extremely sensitive to copper. At a concentration of 0.5 mg/l, 100-percent mortality took place in 3 days. Using a 0.2 mg/l concentration at 10 and 20 C, all clams died within 23 days at the lower temperature, while at the higher temperature all succumbed in 6 to 8 days. When 0.1 mg/l Cu at 20 C was used, all animals died in 10 to 12 days. Raymont and Shields (1964) in studies with the marine polychaete worm *Nereis,* showed that a concentration of 1.5 mg/l Cu was lethal in 2 to 3 days, and concentrations exceeding 0.05 mg/l Cu were lethal in approximately 4 days.

Clendenning and North (1958, 1960) and North and Clendenning (1958, 1959) evaluated the effect of copper (from the chloride and sulfate salts) on the rate of photosynthesis of the giant kelp, *Macrocystis pyrifera.* With 0.1 mg/l of copper, net photosynthesis was inhibited by 50 percent in 2 to 5 days and 70 percent in 7 to 9 days. Visible injury appeared in 10 days. Copper was slightly less toxic than mercury but more so than nickel, chromium, lead, or zinc. Marvin, Lansford, and Wheeler (1961) found 0.05 mg/l Cu toxic to *Gymnodinium breve* (red tide organism).

Mercury.—Mercury is found in seawater at a level of 0.00003 mg/l. It is found in marine plants at approximately 0.03 mg/l.

Irukayama (1966) reported on a mercurial pollution incident in Japan, which was first recognized in 1953. A severe neurological disorder resulted in the area of Minamata Bay as a result of eating fish and shellfish from these waters. Many species of animals including waterfowl were succumbing to the "disease" called Minamata disease. Clinical features were cerebellar ataxia, constriction of visual fields, and dysarithia. Pathological findings were regressive changes in the cerebellum and cerebral cortices. Investigation through 1965 suggested that the main cause was the spent factory waste of the Kanose Factory upstream from the Minamata Bay area. Methyl mercury compounds, waste byproducts from the acetaldehyde synthesis process, were being discharged and concentrated especially in shellfish.

Ukeles (1962) made a study of pure cultures of marine phytoplankton in the presence of toxicants. One of the toxic materials used was lignasan (ethyl mercury phosphate) a bactericide-fungicide. She found lignasan to be lethal to all species at 0.06 mg/l and 0.0006 was the highest level used not causing drastic inhibition of growth.

Clendenning and North (1960) and North and Clendenning (1958) found that 0.5 mg/l of mercury added as mercuric chloride caused a 50-percent inactivation of photosynthesis of the giant kelp, *Macrocystis pyrifera*, during a 4-day exposure. A concentration of 0.1 mg/l caused a 15-percent decrease in photosynthesis in 1 day and complete inactivation in 4 days. Mercury was more toxic than copper, hexavalent chromium, zinc, nickel, or lead. For phytoplankton, the minimum lethal concentration of mercury salts has been reported to range from 0.9 to 60 mg/l of mercury (Hueper, 1960). The toxic effects of mercury salts are accentuated by the presence of trace amounts of copper (Corner and Sparrow, 1956).

Lead.—Lead is found as a local pollutant of rivers near mines and from the combustion of leaded gasolines. The lead concentration in seawater is in the order of 0.00003 mg/l. It is found in marine plants at a level of approximately 8.4 mg/l. Residues in marine animals reach a concentration in the range of 0.5 mg/l. It is highest in calcareous tissue.

Wilder (1952) found that lobsters died within 20 days when kept in lead-lined tanks, while in steel-lined and other types of tanks, they survived for 60 days or longer.

North and Clendenning (1958) found that lead was less toxic to the giant kelp, *Macrocystis pyrifera*, than mercury, copper, hexavalent chromium, zinc, or nickel.

Pringle (unpublished data), in studies on the effects of lead on the Eastern oyster, *Crassostrea virginica*, found a 12-week TL_m value of 0.5 mg/l and an 18-week TL_m value of 0.3 mg/l. Concentrations of 0.1 to 0.2 mg/l induced noticeable changes in mantle and gonadal tissue under 12 weeks of exposure.

Nickel.—Nickel is found in sea water in a concentration of about 0.0054 mg/l. Marine plants contain up to 3 mg/l and this may be higher in plankton. Marine animals contain levels in the range of 0.4025 mg/l. Nickel pollution is caused by industrial smoke and other wastes. It is very toxic to most plants but less so to animals. Haydu (unpublished data), in long-term studies with oysters, found that a level of 0.121 mg/l nickel caused considerable mortality.

Zinc.—Zinc is found in sea water in a concentration of 0.01 mg/l. Marine plants may contain up to 150 mg/l of zinc. Marine animals contain zinc in the range of 6 to 1500 mg/l. It is accumulated by some species of coelenterates and mollusks. Speer (1928) reports that very small amounts of zinc are dangerous to oysters.

Clendenning and North (1960) and North and Clendenning (1958) tested the effect of zinc sulfate on the giant kelp, *Macrocystis pyrifera*. Four-day exposure to 1.31 mg/l of zinc showed no appreciable effect on the rate of photosynthesis, but 10 mg/l caused a 50-percent inactivation of kelp.

Other Toxicants

Ammonia-ammonium compounds.—Ammonia is found in the discharge of many industrial wastes. It has been shown that at a level of 1.0 mg/l NH_3, the ability of hemoglobin to combine with oxygen is impaired and fish may suffocate. Evidence indicates that ammonia exerts a considerable toxic effect on all aquatic life within a range of less than 1.0 mg/l to 25 mg/l, depending on the pH and dissolved oxygen level present.

Cyanides.—Hydrocyanic acid or hydrogen cyanide and its salts, the cyanides, are important industrial chemicals. The acid and its salts are extremely poisonous.

Hydrogen cyanide is largely dissociated at pH levels above 8.2 and its toxicity increases with a decrease in pH. The toxic action of cyanides increases rapidly with a rise in temperature.

Fish can recover from short exposure to concentrations of less than 1.0 mg/l (which seems to act as an anaesthetic) when removed to water free of cyanide. They appear to be able to convert cyanide to thiocyanate, an ion that is not inhibitory on the respiratory enzymes. Complex cyanides formed by the reaction of CN with zinc or cadmium are much more toxic. However, the reaction between CN and nickel produces a cyanide complex less toxic than the CN itself at high pH levels.

Sulfides.—Sulfides in water are a result of the natural processes of decomposition, sewage, and industrial wastes such as those from oil refineries, tanneries, pulp and paper mills, textile mills, chemical plants, and gas manufacturing facilities. Most toxicity data available are based on fresh water fish. Concentrations in the range of less than 1.0 mg/l to 25.0 mg/l are lethal in 1 to 3 days.

Fluorides.—Fluorides are present in varying amounts in the earth's crust. They are used as insecticides as well as in water treatment and many other uses. While normally not present in industrial wastes, they may be present in trace or higher concentrations due to spillage. Data in fresh water indicate that they are toxic to fish at concentrations higher than 1.5 mg/l.

Detergents and surfactants.—During the past twenty years, synthetic detergents have replaced a majority of the soap products. Concern about their importance in pollution was heightened by the visible evidence of their foaming in the Nation's waterways. Their toxicity to the aquatic fauna has been very extensively studied, but for the most part it is difficult to establish safe criteria because of the varying conditions of the tests. Relatively little bioassay work on their effects on marine biota has been published, but it is indicated that, unlike soap, detergents are more toxic in highly saline water than they would be in the fresh water areas of tidal estuaries (Eisler, 1965; Eisler and Derrel, 1966).

The 96-hour TL_m values of an ABS detergent to five species of marine fish ranged from 7 to 22 mg/l (Eisler, 1965). Marine kelp were more sensitive and photosynthesis was inhibited 50 percent after 96-hour exposures to about 1.0 mg/l.

Pathogenic organisms.—Oysters, clams, and mussels have a demonstrated ability to accumulate microorganisms, including bacteria and viruses, from their aquatic environments and to serve as a vehicle for the transmission of these microorganisms to their consumers (U.S. DHEW, 1956, 1958, 1962, 1965a; Liu, et al., 1967).

Controls to prevent the transmission of disease through this route have been provided in the United States through the National Shellfish Sanitation Program (NSSP) administered by the Public Health Service, Department of Health, Education, and Welfare on the behalf of the interested State and Federal agencies and the shellfish industry (1965b). This program has established bacteriological quality standards for those waters from which shellfish are to be harvested for direct marketing. These standards, as described in the NSSP Manual of Operation, should be observed for those estuarine areas used for commercial production of shellfish for direct marketing (U.S. DHEW, 1965). The standards that are applied to shellfish harvesting areas have been revised periodically through the mechanism of a shellfish sanitation workshop held at 2 or 3-year intervals. As these standards are revised so should the water quality criteria be modified.

Tar, gas, and coke wastes.—The distillation of coal for the production of gas, coke, and tarry materials used in the manufacture of dyes and various organic chemicals results in a watery waste known as ammoniacal gas liquor, the disposal of which can cause detrimental effects. Ammoniacal gas liquor contains free ammonia, ammonium salts, cyanide, sulfide, thiocyanate, and a variety of aromatic compounds including pyridine, phenols, cresols, xylenols, and aromatic acids. After treatment to remove ammonia, the waste is called "spent gas liquor." Phenol or carboxylic acid is the most abundant of its many phenolic substances, probably the most dangerous to fish.

Phenolic substances are also present in materials used in road surfacing, sheep dips, and many industrial wastes such as those associated with the manufacture of plastics, dyes, and disinfectants. Gas liquor, discharged untreated to a stream, has an extremely high oxygen demand, many times greater than that of sewage. These various groups of organic substances produce a variety of effects on fish varying from intoxication and anaesthesia to paralysis and death.

Pure compounds representative of these groups found in such coal tar wastes have been shown to be toxic in ranges of 2 to 75 mg/l for cresols and 0.1 to 50 mg/l for phenols, for fresh water fish and lower aquatic life.

Petroleum refining and petrochemical wastes.—The volatile components of petroleum consist mainly of aliphatic hydrocarbons. In addition to paraffins and olefins, some petroleums contain relatively high percentages of naphthenes and aromatic hydrocarbons. The less volatile fractions of petroleum are used as fuels, lubricants, and construction materials (asphalt). These substances are somewhat more irritating to the skin and some are carcinogenic, but less so than coal tar products.

Pulp and paper manufacturing wastes.—The types of pulp produced and pulping technology have undergone considerable change in the last 20 years and the trend continues. Modern pulpmills are geared to produce a variety of pulp grades due to the increasing demands for specialty products. The characteristics of the waste waters from these specialty pulp grades can vary considerably. An example of this can be seen in the BOD loadings of the following sulfite grade pulps produced in a west coast mill:

Paper making—130 lb BOD/ADT (air dry ton).
Alpha hardwood—300 lb/ADT.
FAC–SAC—450 lb/ADT.

The major pulping processes include kraft, sulfite, semichemical, and nonchemical such as groundwood. The kraft process accounts for approximately 75 percent of the total pulp production in the United States. The number of mills using the sulfite process are declining, some are being converted to the kraft process.

From the standpoint of water pollution, kraft and sulfite mills are of great significance. The principal problems associated with pulpmill wastes are toxicity, depressed DO's, and slime growths. Clear-cut cases of acute toxicity attributable to pulpmill wastes in modern times seldom exist except when spills or other accidents occur. It is much more common to encounter problems related to slime growths, depressed DO's, and to long-term or chronic effects on the biota.

A substantial portion of pulpmill wastes including the toxic components are very amenable to microbial degradation. In one study, kraft mill wastes were found to be nontoxic to oysters at a dilution of 1:20 when the BOD of the waste was reduced by 80 percent employing biological treatment. In a similar study, the toxicity of kraft wastes to silver salmon was found to diminish proportionally to the degree of BOD reduction above 50 percent, again using biological treatment. The results of a recent study by scientists of the International Pacific Salmon Commission indicate a fairly close relationship between BOD reduction and decrease in the toxicity of kraft wastes. They found no apparent toxicity to salmon when the BOD was reduced by 65 percent. While similar studies have not been made with sulfite liquors, there is some evidence that the toxic components of this waste are also degradable. It is important to recognize that the biological mechanism or degradation involved in secondary treatment is essentially similar to that in receiving waters. Given sufficient time, the process of degradation of the toxic components of pulp wastes also take place in receiving waters.

Because of the great complexity and variability of pulpmill wastes, it is difficult to find a satisfactory expression for concentration. Attempts have been made to relate toxicity to BOD, COD, total solids, PBI (Pearl Benson Index—a measure of the lignin content of pulp wastes), and various reference animals. There is a general relationship with all of these criteria; i.e., the higher the values, the greater the toxicity. Pulpmill dosages or dilutions have been used in bioassays on the basis of applied initial BOD. The response of the test animals has been found to vary considerably to given concentrations of applied BOD even from the wastes of the same mill. This would indicate that the concentration of toxicants in the total biologically amenable fraction is subject to considerable variation. This would not only explain the lack of a good relationship between the toxicity and initial BOD, but it would also explain why, on the other hand, there can coexist a good relationship between BOD reduction and reduction in toxicity. The latter is subject to degradation regardless of the proportions of toxicants and the other to biodegradable substances.

The shortcomings of BOD as an expression of the concentration of toxicity would seem to be equally applicable to the PBI test. This test has been recommended as a measure of SWL (sulfite waste liquor) concentration. It measures the lignins in SWL which constitute an appropriate substance for tracing in receiving waters and for analysis due to their stability and high concentrations. As indicated earlier, critical tests to determine the relationship between BOD reduction and reduction in toxicity have not been conducted with SWL. Nevertheless, there is sufficient evidence to indicate that the toxic components of SWL also reside in the biodegradable fraction and are also degradable. The composition of SWL in receiving waters at different distances from the point of discharge would therefore differ even though similar PBI values may occur. The toxicity of fresh SWL at a PBI concentration of 50 mg/l would be much greater than of biologically stabilized SWL at the same PBI concentration. There is clear indication that further study of SWL toxicity and biodegradation is necessary.

The toxicity of kraft and sulfite wastes to aquatic life is amply reported in the literature. Deleterious effects produced by SWL (generally considered less toxic than kraft wastes) are reported from PBI values as low as 2.0 mg/l for oyster larvae to concentrations greater than 1,000 mg/l for the adult clams *Mya* and *Macoma*. Long-term bioassays with Pacific and Kumamoto oysters, carried out at Oregon State University using calcium-base SWL (10 percent solids), showed no adverse effects at 50 mg/l after 266 days of exposure. Slightly deleterious effects were noted at 100 mg/l, indicating maximum safe limits lie between 50 to 100 mg/l. Continuing field studies at Grays Harbor, Wash., support these findings. In bioassays conducted in salt water by the Washington State Department of Fisheries, salmon exposed for 30 days to concentrations of approximately 500 mg/l of 10-percent SWL showed no apparent ill effects. Herring eggs, on the other hand, were adversely affected at concentrations greater than 96 mg/l.

The apparent tolerance level for salmon in salt water using kraft wastes was found by the above investigation to range from dilutions of 1:16 to 1:90 after 14 to 30 days of exposure. Growth studies conducted at Oregon State University by the National Council for Stream Improvement using raw kraft wastes in fresh water showed no adverse effects to salmonid fishes after 3 to 5 weeks exposure in dilutions of 1:100. English (in press), in his field studies of the English sole in Puget Sound, reports a sustained and thriving fishery in an area affected by SWL. Recent work by the Federal Water Pollution Control Administration (USDI 1967a) in Puget Sound showed damage to oyster larvae and developing English sole eggs at concentrations greater than 10 mg/l of 10-percent SWL. According to this report, oyster growth and market condition is adversely affected and phytoplankton productivity is inhibited at SWL concentrations over 50 mg/l.

Determining the toxicity of complex wastes like oil, refinery petrochemicals, and pulpmill wastes presents a number of problems. For one thing, they contain many known and, perhaps, equally as many unknown toxic substances in small quantities. The toxicological and other physical and chemical characteristics can vary considerably during any given day, in any given plant, due to changes in processes, sources of supplies, and the end product being produced. Considerable variation in effluent characteristics can occur even in a 1-day period. The resulting wastes from these industries contain upwards of several hundreds of compounds representing a number of homologous series of compounds from different organic groups. This complexity is augmented by the treatment of the wastes, as well as by the spectrum of products manufactured from the complex starting material used. The relative ability to react biochemically and to exert an oxygen demand is characteristic of organic materials of such primary significance.

Many groups or series of compounds indicated to be present in such wastes have been shown to be toxic in varying degrees to aquatic life. It is extremely difficult at this time, however, to place a concentration limit or set threshold criteria for such complex systems and hence should be individually bioassayed and their discharge managed accordingly.

Waterfront and boating activities.—Increasing activities by commercial, military, and recreational vessel operators raise the specter of introduction of toxic materials in quantities sufficient to affect marine organisms adversely. This is particularly likely in the case of confined waters of small tidal tributaries, lagoons, embayments, and other marine areas employed as harbors.

Toxic materials are used to prevent activities of boring and fouling marine organisms. Usually, however, every effort is bent in the case of toxic coatings to prevent rapid release of toxic materials into the environment since rapid loss reduces effectiveness of such coatings and increases costs. Some leaching is unavoidable—even necessary. Thus, the presence in confined harbors of many vessels whose bottoms are coated with toxic materials already presents hazards in some places. This would be especially true after spring "fitting out" for small boats.

Boatowners, boat and boatyard operators, fishing and commercial pier and marina operators are not especially noted for the care extended to nearby waters. Commonly, everything that can be is flushed or jettisoned into the water. Purposeful discharges are many—though perhaps decreasing as emphasis on water pollution has increased. Paint leaching, paint spillage, oil and gasoline spillage, detergents, wood preservatives, exhausted containers, metallic objects of all types (zinc, copper, brass, iron, etc.), and other jetsam contribute to contamination from these sources.

Except for confined areas where there are many of these operations such as large shipyards, major military and commercial anchorages, and large and small boat anchorages, it is doubtful that toxicity from these operations is of serious proportions in tidal waters at this point. As with other fouling or contaminating activities of society, however, efforts should be made to keep biological damage from these sources to a minimum. Some discharges are controllable and should fall under the same rules as industrial or community discharges. In the case of large marinas, shipyards, or major anchorages, requirements suggested elsewhere may have to be applied. Future research should include specific attention to this aspect.

Similar comments can be made about waterfront structures and port operations. There is considerable use of toxic materials in preservation of wood, steel, and masonry structures used on marine waterfronts. Discharge of toxic materials, surfactants, petroleum products, other materials and jetsam is common. Similar recommendations can be made for control and research as those for boat, boatyard, and vessel operations.

Disposal of laboratory wastes.—The rapid growth of marine sciences during the past decade is reflected in an ever-increasing number of stations and laboratories engaged in the study of various aspects of oceanography. These institutions are located along the entire coastline of the United States: 28 on the Atlantic, 12 on the Gulf, and 29 on the Pacific. About 2,500 persons (investigators, students, technicians, and laboratory assistants) are employed in these 66 establishments (Hiatt, 1963).

The above number includes institutions operated by Federal and State governments, by universities or privately endowed concerns which receive their main support from the government and national foundations. Other laboratories, hospitals, and research institutions operated by industrial concerns for their specific needs are not included in this total. The laboratories range from small establishments, with less than four investigators, to very large institutions employing or providing research space for 200 to 500 investigators.

The types of research cover various fields of biology, microbiology, experimental physiology, biochemistry, chemistry, biophysics, molecular biology, radiobiology, fishery biology, fishery management, and industrial research. Consequently, the effluents discharged into estuarine and coastal waters vary from ordinary household sewage to mixtures containing an array of organic and inorganic compounds, drugs, and radioactive isotopes. The composition of these effluents cannot be predicted with certainty because the type of research varies greatly from year to year. The laboratory effluent is separated usually from the sea water system, which as a rule has independent plumbing, but is mixed with the domestic sewage and frequently is discharged into natural waters. When many scientific establishments are concentrated in a relatively small area, the situation may become serious. Chlorinated raw sewage entering the harbor a short distance from shore may be caught by a tidal eddy and for several hours circulate close to the sea water intakes of several laboratories before it is carried out by tides.

To maintain desired water quality requirements for aquatic life, it is necessary to separate laboratory effluents from domestic sewage and provide treatment that renders them harmless to aquatic biota. Under no conditions should highly toxic chemical compounds or drugs be permitted to be discharged into natural waters if toxic concentrations of them can be detected by chemical and physical methods.

Many marine laboratories are utilizing exotic and endemic microorganisms, some pathogenic, in research. Extreme caution must be exercised to prevent contamination of water by introduction of biological materials which can harm marine organisms.

Laboratory administrators should be responsible for the periodical examination of the toxicity of the effluent discharged into natural waters by their institutions.

Recommendation: (1) Allowable concentrations of metals, ammonia, cyanides, and sulfides should be determined by the use of 96-hour TL_m values and appropriate application factors. Preferably, the TL_m values should be determined by flow-through bioassays in which environmental factors are maintained at levels under which these materials are most toxic. Tests should utilize the most sensitive life stage of species of ecological or economic importance in the area. Tentatively, it is suggested that application factors should be 1/100 for pesticides and metals, 1/20 for ammonia, 1/10 for cyanide, and 1/20 for sulfides.

(2) There is evidence that fluorides are accumulative in organisms. It is tentatively suggested that allowable levels should not exceed those for drinking water.

(3) The further dilution of wastes in marine waters suggests that the adoption of criteria established for detergents and surfactants in fresh water also will protect adequately biota in the marine environment.

(4) Bacteriological criteria of estuarine waters ultilized for shellfish cultivation and harvesting should conform with the standards as described in the National Shellfish Sanitation Program Manual of Operation. These standards provide that:

(a) Examinations shall be conducted in accordance with the American Public Health Association recommended procedures for the examination of sea water and shellfish.

(b) There shall be no direct discharges of untreated sewage.

(c) Samples of water for bacteriological examination to be collected under those conditions of time and tide which produce maximum concentration of bacteria.

(d) The coliform median MPN of the water does not exceed 70/100 ml, and not more than 10 percent of the samples ordinarily exceed an MPN of 230/100 ml for a 5-tube decimal dilution test (or 330/100 ml where the 3-tube decimal dilution test is used) in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions.

(e) The reliability of nearby waste treatment plants shall be considered in the approval of areas for direct harvesting.

(5) It is also essential to monitor continuously waste from tar, gas, and coke, petroleum refinery, petrochemical, and pulp and paper mill operations. They all produce complex wastes of great variability, not only from facility to facility, but also from day to day. This should be done on an individual basis with bioassays. These tests should be made at frequent intervals to determine TL_m values as described for other wastes. For the more persistant toxicants, an application factor of 1/100 should be used while for unstable or biodegradable materials an application of 1/20 is tentatively suggested.

(6) Concentration of other materials with noncumulative toxic effects should not exceed 1/10 of the 96-hour TL_m value. For toxicants with cumulative effects, the concentrations should not exceed 1/20 and 1/100 for the above respective values.

When two or more toxic materials that have additive effects are present at the same time in the receiving water, some reduction in the permissible concentrations as derived from bioassays on individual substances is necessary. The amount of reduction required is a function of both the number of toxic materials present and their concentrations with respect to the derived permissible concentration. An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible combination for the mixture is through the use of following relationship:

$$\left(\frac{C_a}{L_a}+\frac{C_b}{L_b}\cdots+\frac{C_n}{L_n}\leq 1\right)$$

Where C_a, C_b . . . C_n are the measured concentrations of the several toxic materials in the water and L_a, L_b . . . L_n are the respective permissible concentrations (limits) derived for the materials on an individual basis. Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary.

literature cited

(*1*) Albersmeyer, W., and L. V. Erichsen. 1959. Investigations on the effects of tar constituents in waste waters. Z. Fisch. 8(1/3): 29–65.

(*2*) Allee, W. C., A. E. Emerson, O. Park, T. Park, and K. P. Schmidt. 1949. Principles of animal ecology. W. B. Saunders Co. Philadelphia. 837 pp.

(*3*) American Fisheries Society. 1960. A list of common and scientific names of fishes from the United States and Canada. 2d ed. Spec. Publ. No. 2. 102 pp.

(*4*) American Petroleum Institute. 1949. Waste water containing oil. *In:* Manual of Refinery Wastes, Sect. 1, 4th ed.

(*5*) American Society of Civil Engineers. Committee on Thermal Pollution, Sanitary Engineering Division. 1967. Bibliography on thermal pollution. J. San. Eng. Div., Amer. Soc. Civil Eng., Proc. 93(SA 3): 85–113.

(*6*) American Society for Testing and Materials. Tentative method of test for evaluating inhibitory toxicity of industrial waste waters. ASTM Standards, par. 23: 517–525.

(*7*) Anderson, B. G. 1950. The apparent thresholds of toxicity to *Daphnia magna* for chlorides of various metals when added to Lake Erie water. Amer. Fish. Soc. Trans. 78: 96.

(*8*) Andrews, J. D. 1964. Effects of river flow regulation by Salem Church Dam on marine organisms. Va. Inst. Mar. Sci. Spec. Scientific Rep. Appl. Mar. Sci. Ocean Eng. No. 4. 20 p.

(*9*) Ahi, S. M., and W. L. Powers. 1938. Salt tolerances of plants at various temperatures. Plant Physiol. 13: 767–789.

(*10*) Bandt, H. J. 1955. Fischereischaden durch phenolabwasser. Wasserwirtsch.-Wassertech. 9: 1.

(*11*) Bardach, J. E., M. Fujiya, and A. Holl. 1965. Detergents: Effects on the chemical senses of the fish *Ictalurus natalis* (leSueur). Science 148: 1605.

(*12*) Barlow, J. P. 1955. Physical and biological processes determining the distribution of zooplankton in a tidal estuary. Biol. Bull. 109(2): 211–225.

(*13*) Bartholemew, G. W., and R. E. MacMillan. 1961. Water economy of the California quail and its use of sea water. The Auk. 78(4): 505–514.

(*14*) Berry, A. E. 1951. Survey of industrial wastes in the Lake Huron-Lake Erie Section of the International Boundary Waters. Sew. Ind. Wastes 23(4): 508–538.

(*15*) Boetius, J. 1954. Foul taste of fish and oysters caused by chlorophenol. Medd. Denmarks Fishlog Havundersdg. N.S. 1: 1–8.

(*16*) Bourn, W. S. 1932. Ecological and physiological studies on certain aquatic angiosperms. Boyce Thompson Inst. Contrib. 4: 425–496.

(*17*) Bousfield, E. L. 1955. Ecological control of the occurrence of barnacles in the Miramichi estuary. Natl. Mus. Canada Bull. No. 137: 69 p.

(*18*) Bowden, K. F. 1963. The mixing processes in a tidal estuary. Int. J. Air Water Poll. 7: 343–356.

(*19*) Brett, J. R. 1960. Thermal requirements of fish—three decades of study, 1940–70. pp. 110–117. *In:* Biological Problems in Water Pollution. 1959 seminar, Trans. PHS Tech. Rep. W60-3. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.)

(*20*) Bringmann, G., and R. Kuhn. 1959a. The toxic effects of waste water on aquatic bacteria, algae, and small crustaceans. Gesundheits-Ing. 80: 115.

(*21*) Bringmann, G., and R. Kuhn. 1959b. Water toxicology studies with protozoans as test organisms Gesundheits-Ing. 80: 239.

(*22*) Brooks, R. R., and M. G. Rumsby. 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr. 10: 521–527.

(*23*) Brungs, W. A. Effect of exposure of zinc on minnow reproduction. *In press.*

(*24*) Brungs, W. A., and D. I. Mount. 1967. A device for continuous treatment of fish in holding chambers. Amer. Fish. Soc., Trans. 96(1): 55–57.

(*25*) Buck, D. H. 1956. Effects of turbidity on fish and fishing. p. 249. *In:* 21st North Amer. Wildlife Conf. Trans.

(*26*) Burdick, G. E., and M. Lipschuetz. 1950. Toxicity of ferro- and ferri-cyanide solutions to fish and determination of the cause of mortality. Amer. Fish. Soc. Trans. 78: 192.

(*27*) Butler, P. A. 1954. Selective setting of oyster larvae on artificial cultch., Natl. Shellfish Assoc., Proc. 45: 95–105.

(*28*) Butler, P. A. 1965. Reaction of some estuarine mollusks to environmental factors. pp.92–104. *In:* Biological problems in water pollution, 3d seminar, 1962. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.)

(*29*) Butler, P. A. 1966a. Fixation of DDT in estuaries. pp. 184–189. *In:* 31st No. Am. Wildl. and Nat. Resources Conference, Trans. Mar. 14, 15, and 16, 1966.

(*30*) Butler, P. A. 1966b. Pesticides in the environment and their effects on wildlife. J. Appl. Ecol. 3 (Suppl.): 253–259.

(*31*) Cairns, J., Jr. 1956. The effects of increased temperatures upon aquatic organisms. 10th Ind. Waste Conf., Proc. Purdue Univ. Eng. Bull. 40(1): 346.

(*32*) Cairns, J., Jr. 1957. Environment and time in fish toxicity. Industrial Wastes 2: 1. p. 177. *In:* McKee and Wolf, 1963. Water Quality Criteria, 2d edition, State Water Quality Control Board, Sacramento, Calif.

(*33*) Cairns, J., Jr., and A. Scheier. 1957. The effects of temperature and hardness of water upon the toxicity of zinc to the common bluegill (*Lepomis macrochirus* Raf.). Notulae Naturae 1(299): 12.

(*34*) Cairns, J., Jr., and A. Scheier. 1958a. The effect of periodic low oxygen upon the toxicity of various chemicals to aquatic organisms. 12th Ind. Waste Conf. Proc. 1957. Purdue Univ., Lafayette, Ind.

(*35*) Cairns, J., Jr., and A. Scheier. 1958b. The effects of temperature and hardness of water upon the toxicity of zinc to the pond snail, *Physa heterostropha* (Say). Notulae Naturae 308: 11.

(*36*) Cairns, J., Jr., and A. Scheier. 1963a. The acute and chronic effects of standard sodium alkyl benzene sulfonate upon pumpkinseed sunfish, *Lepomis gibbosus* (Linn.), and the bluegill sunfish, *Lepomis macrochirus* (Raf.) 17th Ind. Waste Conf., Proc. Purdue Univ., Ext. Ser 112 (14).

(*37*) Cairns, J., Jr., and A. Scheier. 1963b. Environmental effects upon cyanide toxicity to fish. Notulae Naturae 361.

(*38*) Cairns, J., Jr., and A. Scheier. 1964. The effects of sublethal levels of zinc and of high temperature upon the toxicity of a detergent to the sunfish, *Lepomis gibbosus* (Linn.). Notulae Naturae 367.

(*39*) Cairns, J., Jr., A. Scheier, and N. E. Hess. 1964. The effects of alkyl benzene sulfonate on aquatic organisms. Water Wastes 9(1): 22–28.

(*40*) California Department of Fish and Game and Department of Water Resources. 1963. Delta fish and wildlife protection study. Rep. No. 2. 66 pp.

(*41*) Carriker, M. R. 1967. Ecology of estuarine benthic invertebrates: a perspective. pp. 442–487. *In:* Estuaries. Amer. Assoc. Advance. Sci. Publ. No. 83., Washington, D.C.

(*42*) Carritt, D. E. 1959. Radioactive waste disposal into atlantic and coastal waters. National Academy of Sciences-National Research Council, Publ. No. 655. Washington, D.C. 37 pp.

(*43*) Chipman, W. A., and P. S. Galtsoff. 1949. Effects of oil mixed with carbonized sand on aquatic animals. U.S. Fish and Wildlife Service, Spec. Rep. Fish. No. 1. 52 pp.

(*44*) Chitty, D. 1948. Dispersal of fuel oil from harbor waters. Commonwealth Eng. (New Zealand) 36(5): 214–215.

(*45*) Clarke, G. L. 1947. Poisoning and recovery in barnacles and mussels. Biol. Bull., Woods Hole 92: 73; Water Pollution Abs. 20.

(*46*) Clarke, G. L. 1954. Elements of ecology; John Wiley & Sons. New York 534 pp.

(*47*) Clendenning, K. A., and W. J. North. 1958. The effects of waste discharges on kelp. Quart. Progress Rep. Inst. of Marine Resources, Univ. California, La Jolla, IMR Ref. 58–6.

(*48*) Clendenning, K. A., and W. J. North. 1960. Effects of wastes on the giant kelp, *Macrocystis pyrifera*. p. 82. *In:* 1st Int. Conf. on Waste Disposal in the Marine Environment, Proc. Pergamon Press, N.Y.

(*49*) Cohen, J. M., and C. Pinkerton. 1966. Widespread translocation of pesticides by air transport and rain-out pp. 163-176. *In:* Organic pesticides in the environment. Advances in chemistry levies No. 60. American Chemical Society, Washington, D.C.

(*50*) Committee on the Prevention of Pollution of the Sea by Oil. 1953. Report to the Ministry of Transport, H. M. Stationery Office, London, 50 pp.

(*51*) Corner, E. D. S., and B. W. Sparrow. 1956. The modes of action of toxic agents. I. Observations on the poisoning of certain crustaceans by copper and mercury. J. Mar. Biol. Assoc. United Kingdom 35: 531.

(*52*) Crance, J. H. 1963. The effects of copper sulphate on Microcystis and zooplankton in ponds. Prog. Fish-Cult. 25(4): 198–202.

(*53*) Darnell, R. M. 1967. Organic detritus in relation to the estuarine ecosystem. pp. 376–382. *In:* Estuaries. Amer. Assoc. Advance. Sci. Publ. No. 83. Washington, D.C.

(*54*) Davis, H. C. 1960. Effects of turbidity-producing materials on eggs and larvae of the clam (*Venus/Mercenaria mercenaria*). Biol. Bull. 118 (1): 48–54.

(*55*) Doll, E. R., F. E. Hull, and W. M. Luskar, Jr. 1946. Toxicity of sodium chloride solution for baby chicks. Veterinary Med. 41(10): 361–363.

(*56*) Dorris, T. C., W. Gould, and C. R. Jenkins. 1960. Toxicity bioassay of oil refinery effluents in Oklahoma. pp. 276–285. *In:* Biological problems in water pollution. 1959 Seminar, Trans. PHS Tech. Report W60–3. (Rebert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.)

(*57*) Doudoroff, P., et al. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes for fish. Sew. Ind. Wastes 23(11): 1380–1397.

(*58*) Doudoroff, P. 1956. Some experiments on the toxicity of complex cyanides to fish. Sew. Ind. Wastes 28(8): 1020.

(59) Doudoroff, P. 1957. Water quality requirements of fishes and effects of toxic substances. Chap. 9, *In:* M. E. Brown, The physiology of fishes. Vol. 2. (Behavior). Academic Press, Inc. New York, 402 pp.

(60) Doudoroff, P., and M. Katz. 1950. Critical review of literature on the toxicity of industrial wastes and their components to fish. I. Alkalies, acids, and inorganic gasses. II. Metal as salts. Sew. Ind. Wastes 22: 432–458.

(61) Doudoroff, P., and M. Katz. 1953. Critical review of literature on the toxicity of industrial wastes and their components on fish. II. Metal as salts. Sew. Ind. Wastes 25(7): 802–839.

(62) Doudoroff, P., G. Leduc, and C. R. Schneider. 1966. Acute toxicity to fish of solutions containing complex metal cyanides, in relation to concentration of molecular hydrocyanic acid. Amer. Fish. Soc., Trans. 95(1): 6–22.

(63) Doudoroff, P., and P. L. Shumway. 1967. Dissolved oxygen criteria for the protection of fish. Amer. Fish. Soc., Trans. Suppl. to Vol. 96 (Spec. Publ. No. 4): 13–19.

(64) Doudoroff, P., and C. E. Warren. 1962. Dissolved oxygen requirement of fishes. pp. 145–155. *In:* Biological problems in water pollution: 3d Seminar, 1962. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.)

(65) Dugan, P. R. 1967. Influence of chronic exposure to anionic detergents on toxicity of pesticides to goldfish. J. Water Poll. Control Fed. 39(1): 63–71.

(66) Eisler, R. 1965. Some effects of a synthetic detergent on estuarine fishes. Amer. Fish. Soc., Trans. 94(1): 26–31.

(67) Eisler, R., and D. G. Derrel. 1965. Acute toxicity of soaps to estuarine fishes. Prog. Fish-Cult. 26(1): 45–48.

(68) Ellis, M. M. 1934. A photoelectric apparatus for turbidity and light penetration measurement. Sci. 80: 37–38.

(69) Ellis, M. M. 1937. Detection and measurement of stream pollution. U.S. Bur. Fish. Bull. 48(22): 365–437.

(70) Ellis, M. M. 1940. Water conditions affecting aquatic life in Elephant Butte Reservoir. U.S. Bur. Fish. Bull. 34(39): 257–304.

(71) Emery, K. O., and R. E. Stevenson. 1957. Estuaries and lagoons, pp. 673–750. *In:* Hedgpeth, J. W. (ed.), Treatise on marine ecology and paleoecology, Mem. 67, Vol. 1, Geol. Soc. America.

(72) Engelbrecht, R. S., and J. J. Morgan. 1961. Land drainage as a source of phosphorus in Illinois surface waters. pp. 74–79. *In:* Algae and metropolitan wastes. U.S. Public Health Service, SEC TR W61-3.

(73) English, J. N., G. N. McDermott, and C. Henderson. 1963a. Pollutional effects of outboard motor exhaust-laboratory studies. J. Water Poll. Control Fed. 35(7): 923.

(74) English, J. N., E. W. Surber, and G. N. McDermott. 1963b. Pollutional effects of outboard motor exhaust-field studies. J. Water Poll. Control Fed. 35(9): 1121–1132.

(75) English, T. S. Preliminary assessment of the English sole in Port Gardner, Wash. *In press.*

(76) European Inland Fisheries Advisory Committee; Working Party on Water Criteria for European Freshwater Fish. 1964. Water quality criteria for European freshwater fish. Report on finely divided solids and inland fisheries. Tech. Paper No. 1. 21 pp.

(67) Felsing, W. A., Jr. 1966. Proceedings of joint sanitation seminar on North Pacific clams. Alaska Department Health and Welfare, U.S. Department of Health, Education, and Welfare, Washington, pp. 1–34.

(78) Field, H. I., and E. T. Evans. 1946. Acute salt poisoning in poultry. The Veterinary Rec. 38(23): 253–254.

(79) Fitzgerald, G. P., G. C. Gerloff, and F. S. Borg. 1952. Studies on chemicals with selective toxicity to blue-green algae. Sew. Ind. Wastes 24(7): 888–897.

(80) Fry, F. E. J. 1960. The oxygen requirements of fish. pp. 106–109. *In:* Biological problems in water pollution. 1959 Seminar, Trans. PHS Tech. Rep. W60-3. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.)

(81) Fujiya, M. 1960–61. Studies on the effects of copper dissolved in sea water on oysters. Bull. Japan Soc. Sci. Fish. (Japan) 26(5): 462. J. Water Poll. Control Fed. 33: 685.

(82) Galtsoff, P. S. 1932. Life in the ocean, from a biochemical point of view. Wash. Acad. Sci. 22(9): 246.

(83) Galtsoff, P. S. 1948. Red Tide. Spec. Sci. Rep. No. 46, Fish and Wildlife Service. 44 pp.

(84) Galtsoff, P. S. 1949. The mystery of the red tide. Sci. Monthly 68(2): 108–117.

(85) Galtsoff, P. S. 1964. The American Oyster, *Crassostrea virginica*, Gmelin. Fish and Wildlife Service Fish. Bull. 64: 1–480.

(86) Galtsoff, P. S., W. A. Chipman, Jr., J. B. Engle, and H. N. Calderwood. 1947. Ecological and physiological studies of the effect of sulfate pulpmill wastes on oysters in the York River, Va. Fish and Wildlife Service Fish. Bull. 43(51): 59–186.

(87) Galtsoff, P. S., H. F. Prytherch, R. O. Smith, and V. Koehring. 1935. Effects of crude oil pollution on oysters in Louisiana waters. Bull. Bur. Fish. 48(18): 209.

(88) Galtsoff, P. S., and D. V. Whipple. 1931. Oxygen consumption of normal and green oysters. Bull. Bur. Fish. 46: 489–508.

(89) Garrett, W. D. 1962. Collection of slick-forming material from the sea. U.S. Naval Res. Lab. Rep. 5761.

(90) Garrett, W. D. 1964. The organic chemical composition of the ocean surface. U.S. Naval Res. Lab. Rep. 6201.

(91) Geckler, J. R., K. M. Machenthun, and W. M. Ingram. 1963. Glossary of commonly used biological and related terms in water and waste control. USDHEW. Public Health Service Publ. No. 999-WP-2. 22 pp.

(92) Godzch, S. 1961. The toxic effect of Nekal BXG on *Daphnia magna*. Acta Hydrobiol. 3 (4): 281.

(93) Gorham, P. H. 1964. Toxic algae as a public health hazard. J. Amer. Water Works Assoc. 56(11): 1481–1488.

(94) Grande, M 1966. Effect of copper and zinc

on salmonid fishes. 3d Int. Conf. on Water Poll. Res. Messegelande, theresienhohe, Munich, Germany. Sec. I, Paper No. 5.

(95) Griffith, W. H., Jr., 1962–63. Salt as a possible limiting factor to the Suisun Marsh pheasant population. Annual Report, Delta Fish and Wildlife Protection Study, Cooperative Study of California.

(96) Hargis, W. J., Jr. 1965. Multidisciplinary research on an estuarine engineering project. pp. 45–46. *In:* Seminar on multidisciplinary research as an aid to public policy formation. V.P.I. Water Resources Res. Center Bull. No. 2.

(97) Hargis, W. J., Jr. 1966. An evaluation of physical and biological effects of the proposed James River Navigation Project. Va. Inst. Mar. Sci. Spec. Sci. Rep. Appl. Mar. Sci. Ocean Eng. No. 7. 73 pp.

(98) Hartung, R. 1965. Some effects of oiling on reproduction of ducks. J. Wildlife Manage. 29(4): 872–874.

(99) Hassall, K. A. 1962. A specific effect of the respiration of *Chlorella vulgaris.* Nature (Lond.) 193: 90.

(100) Henderson, C. 1957. Application factors to be applied to bioassays for the safe disposal of toxic wastes. Biol. prob. in water pollution. U.S. Public Health Service. pp. 31–37.

(101) Henderson, C., and Q. H. Pickering. 1963. Use of fish in the detection of contaminants in water supplies. J. Amer. Water Works Assoc. 55(6): 715–720.

(102) Henderson, C., Q. H. Pickering, and J. M. Cohen. 1959. The toxicity of synthetic detergents and soaps to fish. Sew. Ind. Wastes 31 (3): 295–306.

(103) Henderson, C., Q. H. Pickering, and A. E. Lemke. 1961. The effect of some organic cyanides (nitriles) on fish. 15th Purdue Ind. Waste Conf. Eng. Bull. of Purdue Univ. Series No. 106. 45(2): 120.

(104) Herbert, D. W. M., and G. C. Merkens. 1961. The effect of suspended mineral solids on the survival of trout. Int. J. Air Water Poll. 5: 46–55..

(105) Herbert, D. W. M., and D. S. Shurben. 1936a. A preliminary study of the effect of physical activity on the resistance of rainbow trout to two poisons. Ann. Appl. Biol. 52: 321–326.

(106) Herbert, D. W. M., and D. S. Shurben. 1936b. The toxicity to fish of mixtures of poison, I. Salts of ammonia and zinc. Ann. Appl. Biol. 53: 33.

(107) Herbert, D. W. M., and J. Vandyke. 1964. The toxicity to fish of mixtures of poisons. II. Copper-ammonia and zinc-phenol mixtures. Ann. Appl. Biol. 53: 415–421.

(108) Herbert, D. W. M., and A. C. Wakeford. 1964. The susceptibility of salmonid fish to poisons under estuarine conditions. I. Zinc sulphate. Int. J. Air Water Poll. 8: 251–256.

(109) Hervey, R. K. 1949. Effect of Chromium on the growth of unicellular Chlorophyceae and diatoms. Bot. Gaz. 111(1): 1–11.

(110) Hiatt, R. W. 1963. (Ed.) World directory of hydrobiological and fishery institutions. Amer. Inst. Biol. Sci., Washington, D.C., 320 pp.

(111) Hickey, J. J., J. A. Keith, and F. B. Coon. 1966. An exploration of pesticides in a Lake Michigan ecosystem. J. Appl. Ecol. 3: 141–154.

(112) Holland, G. Z., G. A. Lasater, E. D. Neumann, and W. E. Eldridge. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. State of Washington, Department of Fish., Res. Bull. 5.

(113) Holland, H. T., D. L. Coppage, and P. A. Butler. 1967. Use of fish brain acetylcholinesterase to monitor pollution by organophosphorus pesticides. Bull. of Environmental Contamination and Toxicol. 2(3): 156–162.

(114) Hublou, W. F., J. W. Wood, and E. R. Jefferies. 1954. The toxicity of zinc or cadmium for chinook salmon. Ore. Fish Comm. Briefs 5(1): 1–7.

(115) Hueper, W. C. 1960. Cancer hazards from natural and artificial water pollutants. Conf. Physiol. Aspects of Water Quality, Proc. USPHS, Washington. 181 pp.

(116) Hutchinson, G. E. 1957. A Treatise on limnology. Vol. I. John Wiley & Sons, Inc. New York. 1015 pp.

(117) Irukayama, K. 1966. The pollution of Minamata Bay and Minamata disease. Advance Water Poll. Res. 3: 153–180.

(118) Isaacs, J. D. 1962. Disposal of low-level radioactive waste into Pacific coastal waters. National Academy of Sciences-National Research Council, Pub. No. 985, Washington, D.C. 87 pp.

(119) Jackson, H. W., and W. A. Brungs, Jr. 1966. Biomonitoring of industrial effluents. 21st Purdue Ind. Waste Conf., Proc. May 3–5, 1966, Pt. I 50(2): 117–124 (March 1967), Eng. Bull., Purdue Univ., Eng. Ext. Series No. 121.

(120) Jensen, W. I., and C. S. Williams. 1964. Botulism and fowl chlorea. pp. 333–341. *In:* Waterfowl tomorrow, Bur. Sport Fish and Wildlife. Superintendent of Documents U.S. Government Printing Office, Washington, D.C.

(121) Jones, J. R. E. 1938. The relative toxicity of salts of lead, zinc and copper to the stickleback (*Gasterosteus aculeatus* L.) and the effect of calcium on the toxicity of lead and zinc salts. J. Exp. Biol. 15: 394–407.

(122) Jordan, I. S., B. E. Day, and R. T. Hendrixson. 1962. Chemical control of filamentous green algae. Hilgardia 32(9): 432–441.

(123) Juday, L., E. A. Birge, G. J. Kemmerer, and R. J. Robinson. 1927. Phosphorus content of lake waters of Northeastern Wisconsin. Wisconsin Acad. Sci., Arts and Letters, Trans. 23: 233–248.

(124) Kalugina, A. A., N. Y. Milovidova, T. V. Sviridova, and I. V. Uralskaya. 1967. Effect of pollution on marine organisms of Novorossiysk Bay of the Black Sea. Hydrobiol. 3(1): 47–53. (In Russian; English Résumé). Kiev, U.S.S.R.

(125) Kauship, D. K. 1963. The influence of salinity on the growth reproduction of marsh plants. Ph.D. Thesis, Utah State Univ. 123 pp.

(126) Keith, J. A. 1966. Reproduction in a population of herring gulls (*Larus argentatus*) contaminated with DDT. J. Appl. Ecol. 3: 57–70.

(127) Ketchum, B. H. 1950. Hydrographic factors

involved in the dispersion of pollutants introduced into tidal waters. J. Boston Soc. Civil Eng. 37(3): 296–314.

(*128*) Ketchum, B. H. 1951. The flushing of tidal estuaries. Sew. Ind. Wastes 23(2): 198–208.

(*129*) Ketchum, B. H. 1954. Relation between circulation and planktonic populations in estuaries. Ecology 35(2): 191–200.

(*130*) Ketchum, B. H. 1957. Phytoplankton nutrients in estuaries. pp. 329–335. *In:* Estuaries. Amer. Assoc. Advance. Sci. Publ. No. 83, Washington, D.C.

(*131*) Krista, L. M., C. W. Carlson, and O. E. Olsen. 1961. Mortality of chicks as influenced by addition of various NaCl levels to the drinking water. Poultry Sci. 40(4): 938–944.

(*132*) Krumholz, L. A., and R. F. Foster. 1957. Accumulation and retention of radioactivity from fission products and other radiomaterials by fresh-water organisms. National Academy of Sciences—National Research Council, Pub. No. 551, Washington, D.C. 88 pp.

(*133*) Lackey, J. B. 1945. Plankton productivity of certain Southeastern Wisconsin lakes as related to fertilization. II. Productivity. Sew. Works J. 17(4): 795–802.

(*134*) Lalou, C. 1965. Concentration des benzo 3,4-pyrenes par les holothuries de de la region de Villefranches et d'Antibes. pp. 363–366. *In:* Pollution marines par les microorganismes et les produits petroliers. Symposium de Monaco (Avril, 1964).

(*135*) LeClerc, E., and F. Devlaminck. 1952. Natural or synthetic detergents and fish. Bull. cen belge et Document Eaux 17: 165.

(*136*) Lemke, A. E., and D. I. Mount. 1963. Some effects of alkyl benzene sulfonate on the bluegill, *Lepomis macrochirus*. Amer. Fish. Soc., Trans. 92(4): 372.

(*137*) Liu, O. C., H. R. Seraichekas, and B. L. Murphy. 1967. Viral depuration of the Northern quahog. Appl. Microbiol. 15: 307–315.

(*138*) Lloyd, R. 1961a. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdnerii* Richardson). J. Exp. Biol. 38: 447.

(*139*) Lloyd, R. 1961b. The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. 49: 535–538.

(*140*) Loosanoff, V. L. 1962. Effects of turbidity on some larval and adult bivalves. pp. 20–94. *In:* Gulf and Caribbean Fish. Inst. 14th Annual Session, November, 1961.

(*141*) Mackenthun, K. M. 1965. Nitrogen and phosphorus in water. U.S. Department of Health, Education, and Welfare, Public Health Service. 111 pp.

(*142*) Mallet, L. 1965. Pollution par les hydrocarbures en particulier du type benzo 4,4- pyrene des rivages mediterranneens francais et plus specialement de la baie de Villefranche. pp. 325–33. *In:* Pollutions marines par les microorganismes et les produits petroliers. Symposium de Monaco (Avril, 1964).

(*143*) Mallet, L., and J. Sardou. 1965. Recherche de la presence de l'hydrocarbure polybenzenique benzo 3,4-pyrene dans le moillieu planctonique de la region de la baie de Villefranche. pp. 331–334. *In:* Pollutions marines par les microorganismes et les produits petroliers. Symposium de Monaco (Avril, 1964).

(*144*) Maloney, T. E. 1966. Detergent phosphorus effect on algae. J. Water Poll. Control Fed. 38(1): 38–45.

(*145*) Maloney, T. C., and C. M. Palmer. 1956. Toxicity of six chemical compounds to 30 cultures of algae. Water Sew. Works. 103: 509–513.

(*146*) Mann, H., and O. J. Schmid. 1961. The influence of detergents upon sperm, fertilization, and development in the trout. Int. Rev. Hydrobiol. 46(419), Water Poll. Abs. 36 (818).

(*147*) Marvin, K. T., C. M. Lansford, and R. S. Wheeler. 1961. Effects of copper ore on the ecology of lagoon. U.S. Fish and Wildlife Service Fish. Bull. 184: 153–160.

(*148*) Martin, A. C., and F. M. Uhler. 1939. Food of game ducks in the United States and Canada. Res. Rep. 30, U.S. Fish and Wildlife Service. Superintendent of Documents Government Printing Office Washington, D.C.

(*149*) McCallum, G. E. 1964. Clean water and enough of it. pp. 471–478. *In:* Waterfowl tomorrow. Bureau of Sport Fish. and Wildlife. Superintendent of Documents Government Printing Office Washington, D.C.

(*150*) McKee, J. E., and H. W. Wolf. 1963. Water quality criteria. The Resources Agency of California, State Water Quality Control Board, Pub. No. 3–A. 548 pp.

(*151*) McNulty, J. K. 1955. The ecological effects of sewage pollution in Biscayne Bay, Fla.: Sediments and the distribution of benthic and fouling macroorganisms. Bull. Mar. Sci. of the Gulf and Caribbean 11(3): 394–447.

(*152*) Mihursky, J. A., and V. S. Kennedy. 1967. Water temperature criteria to protect aquatic life. pp. 20–32. *In:* A Symposium on water quality criteria to protect aquatic life. Amer. Fish. Soc. Spec. Publ. No. 4.

(*153*) Mount, D. I. Chronic toxicity of copper to fathead minnows. *In press.*

(*154*) Mount, D. I. 1966. The effect of total hardness and pH on acute toxicity of zinc to fish. Int. J. Air Water Poll. 10: 49–56.

(*155*) Mount, D. I. 1967. A method for detecting cadmium poisoning in fish. J. Wild. Mgt. 31(1): 168–172.

(*156*) Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–22.

(*157*) Mount, D. I., and C. E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2,4-D. Amer. Fish. Soc., Trans. 96(2): 185–193.

(*158*) Mount, D. I., and R. E. Warner. 1965. A serial-dilution apparatus for continuous delivery of various concentrations of materials in water. U.S. Public Health Service, Environmental Health Series, WSPC, PHS Publ. 999–WP–23.

(*159*) Nelson, N. F. 1953. Factors in the development and restoration of water fowl habitat at Ogden Bay Refuge, Weber County, Utah. Utah State Fish and Game Department, Federal Aid Division, Publ. No. 6., 87 pp.

(*160*) Nelson, T. C. 1925. Effect of oil pollution on marine and wild life. pp. 171–181. *In:* Appendix V to the report of the U.S. Commissioner of Fisheries for 1925.

(*161*) North, W. J., and K. A. Clendenning. 1958. The effects of waste discharges on kelp. Ann. Prog. Rep., Inst. of Marine Resources, Univ. California, LaJolla, IMR Ref. 58–11.

(*162*) North, W. J., and K. A. Clendenning. 1959. The effects of discharged wastes upon kelp. Ann. Rep. Inst. of Marine Resources, California.

(*163*) North, W. J., M. Neuschul, Jr., and K. A. Clendenning. 1965. Successive biological changes observed in a marine cove exposed to a large spillage of mineral oil. pp. 335–354. *In:* Pollutions marines par les microorganismes et les produits petroliers. Symposium de Monaco (Avril, 1964).

(*164*) Ohio River Valley Water Sanitation Commission, Aquatic Life Advisory Committee. 1956. Aquatic life water quality criteria. 2d progress report. Sew. Ind. Wastes 28(5): 678–690.

(*165*) Ohio River Valley Water Sanitation Commission, Aquatic Life Advisory Committee. 1960. Aquatic life water quality criteria. 3d progress report. J. Water Poll. Control Fed. 32(1): 65–82.

(*166*) Olson, P. A. 1958. Comparative toxicity of Cr(VI) and Cr(III) in salmon. pp. 215–218. *In:* Hanford biological research annual report for 1957. HW–53500, unclassified (Hanford atomic products operation, Richland, Wash.).

(*167*) Olson, P. A., and R. F. Foster. 1956. Effect of chronic exposure to sodium dichromate on young chinook salmon and rainbow trout. pp. 35–47. *In:* Hanford biological research annual report for 1955. HW–41500, unclassified (Hanford atomic products operation, Richland, Wash.).

(*168*) Olson, P. A., and R. F. Foster. 1957. Further studies on the effect of sodium dichromate on juvenile chinook salmon. pp. 214–224, *In:* Hanford biological research annual report for 1956. HW–47500, unclassified (Hanford atomic products operation. Richland, Wash.).

(*169*) Olson, R. A., H. F. Brust, and W. L. Tressler. 1941. Studies of the effects of industrial pollution in the lower Patapsco River area. I. Curtis Bay Region. Chesapeake Biological Laboratory, Solomons Island, Md., Pub. No. 43, 40 pp.

(*170*) Olson, T. A. 1964. Blue-greens. pp. 349–356. *In:* Waterfowl tomorrow, Bureau of Sport Fisheries and Wildlife, Superintendent of Documents U.S. Government Printing Office Washington, D.C.

(*171*) Palmer, C. M. 1957. Evaluation of new algicides for water supply purposes. Taste and Odor Control J. 23(1): 1–4.

(*172*) Pearse, A. S., and G. Gunter. 1957. Salinity. pp. 129–158. *In:* Hedgpeth, J. W. (ed.), Treatise on marine ecology and paleoecology, Mem. 67, Vol. 1, Geol. Soc. America.

(*173*) Pickering, Q. H. 1966. Acute toxicity of alkyl benzene sulfonate and linear alkylate sulfonate to the eggs of the fathead minnow, *Pimephales promelas.* Int. J. Air Water Poll. 10: 385–391.

(*174*) Pickering, Q. H. Effect of chronic exposure to cadmium on minnow reproduction. *In press.*

(*175*) Pickering, Q. H. The effects of dissolved oxygen concentrations upon the toxicity of zinc to the bluegill. *Lepomis machochirus,* Raf. *In press.*

(*176*) Pickering, Q. H., and C. Henderson. 1966a. The acute toxicity of some heavy metals to different species of warm-water fishes. Amer. Fish. Soc., Trans. 91(2): 175–184.

(*177*) Pickering, Q. H., and C. Henderson. 1966b. Acute toxicity of some important petrochemicals to fish. J. Water Poll. Control Fed. 38(9): 1419–1429.

(*178*) Pickering, Q. H., and T. O. Thatcher. Chronic toxicity of LAS to fathead minnows. *In press.*

(*179*) Pickering, Q. H., and W. N. Vigor. 1965. The acute toxicity of zinc to eggs and fry of the fathead minnow. Prog. Fish. Cult. 27(3): 153.

(*180*) Pomeroy, L. R. 1960. Residence time of dissolved phosphate in natural waters. Science 131: 1731–1732.

(*181*) Pringle, B. H. Trace metal accumulation by estuarine molluscs. *In press.*

(*182*) Pringle, B. H. Uptake and concentration of toxic chemicals. I. Trace Metals. *In press.*

(*183*) Pritchard, D. W. 1953. Distribution of oyster larvae in relation to hydrographic conditions. 123–132. *In:* Gulf and Carribbean Fish. Inst. Proc. 5th Annual Session, November, 1952.

(*184*) Pritchard, D. W. 1959. Radioactive waste disposal from nuclear-powered ships. National Academy of Sciences-National Research Council, Pub. No. 658, Washington, D.C. 52 pp.

(*185*) Raney, E. C. 1967. Bibliography. Heated discharges and effects on aquatic life with emphasis on fishes. Division of Biological Sciences, Section of Ecology and Systematics, Fernow Hall, Cornell Univ. Ithaca, N.Y. 90 pp. (mimeo.).

(*186*) Ranson, G. 1927. L'absorption de matieres organiques dissoutes par la surface exterieure du corps chez les animaux aquatiques. These. 249–175. *In:* Annales de L'Inst. Ocean, t. IV, 1927.

(*187*) Raymont, J. E. G., and J. Shields. 1962, 1964. Toxicity of copper and chromium in the marine environment. pp. 275–290. *In:* Recommended procedures for the bacteriological examination of sea water and shellfish. 1962. APHA, New York.

(*188*) Redfield, A. C. 1951. The flushing of harbors and other hydrodynamic problems in coastal waters. pp. 127–135. *In:* Hydrodynamics in modern technology, Hydro. Lab., M.I.T.

(*189*) Rosenberg, M. M., and E. Sess. 1954. Tolerance of growing chickens to solution of Hawaiian salt. World's Poultry J. 10(4): 344–351.

(*190*) Rounsefell, G. A., and W. R. Nelson. 1966. Red-tide research summarized to 1964 including an annotated bibliography. U.S. Department of the Interior, Fish and Wildlife Service, Spec. Sci. Rep. No. 535. 85 pp.

(*191*) Rudd, R. L., and R. E. Genelly. 1956. Pesticides, their use and toxicity in relation to wildlife. State of California Fish and Game Department, Game Bull. No. 7.

(*192*) Ryther, J. H. 1954. The ecology of phytoplankton blooms in Moriches Bay and Great

South Bay, Long Island, N.Y. Biol. Bull. 106: 198–209.

(*193*) Ryther, J. H. 1963. Geographic variations in productivity. pp. 347–480. *In:* The sea. M. N. Hill, editor, Vol. 2, Interscience Publishers, New York. 554 pp.

(*194*) Sawyer, C. N. 1947. Fertilization of lakes by agricultural and urban drainage. J. New England Water Works Assoc. 61(2): 109–127.

(*195*) Schmid, O. J., and H. Mann. 1961. Action of a detergent (dodecyl-benzene sulfonate) on the gills of the trout. Nature (Lond.) 192:(678), Water Poll. Abs. 35(1868).

(*196*) Scrivner, L. H. 1946. Experimental edema and ascites in poults (1 day old turkeys). J. Amer. Veterinary Med. Assoc. 108: 27–32.

(*197*) Selye, H. 1943. Production of nephrosclerosis in the fowl by sodium chloride. J. Amer. Veterinary Med. Assoc. 103(798): 140–143.

(*198*) Seydel, E. 1913. Ueber die Wirkung von Mineralölen auf Fischwasser. Mitteilungen d'Fisherei-Vereins für die Provinz Brandenburg 5(3): 26–28.

(*199*) Sierp, F., and H. Theile. 1954. Influence of surface-active substances on sewage treatment and on self-purification in streams. Vom Wasser 21: 197.

(*200*) Skidmore, J. F. 1964. Toxicity of zinc compounds to aquatic animals, with special reference to fish. Quart. Rev. Biol. 39(3): 227–248.

(*201*) Smith, O. R. 1940. Placer mining silt and its relation to salmon and trout on the Pacific coast. Amer. Fish. Soc. Trans. 69: 225.

(*202*) Speer, C. J. 1928. Sanitary engineering aspects of shellfish pollution. Maryland State Department of Health, Bull. No. 3, April, 1928.

(*203*) Sprague, J. B. 1964a. Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Water Poll. Control Fed. 36(8): 990–1004.

(*204*) Sprague, J. B. 1964b. Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Bd. Canada 21(1): 17.

(*205*) Sprague, J. B., and B. A. Ramsay. 1965. Lethal levels of mixed copper-zinc solution for juvenile salmon. J. Fish. Res. Bd. Canada 22 (2): 425.

(*206*) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTE WATER INCLUDING BOTTOM SEDIMENT AND SLUDGES. 1965. 12th Ed. APHA, Inc., New York.

(*207*) Stommel, H. 1953a. Computation of pollution in a vertically mixed estuary. Sew. Ind. Wastes 25: 1065–1071.

(*208*) Stommel, H. 1953b. The role of density currents in estuaries. pp. 305–312. *In:* Part 3, Density currents. Int. Assoc. Hydraulic Res. and Hydraulics Division, Amer. Soc. Civil Eng., Proc. Minnesota Int. Hydraulics Convention.

(*209*) Surber, E. W., J. N. English, and G. N. McDermott. 1965. Tainting of fish by outboard motor exhaust wastes as related to gas and oil consumption. pp. 170–176. *In:* Biological problems in water pollution, 3d seminar, Aug. 13–17, 1962. U.S. Department of Health, Education, and Welfare. Pub. 999–WP–25.

(*210*) Surber, E. W., and O. L. Meehan. 1931. Lethal concentrations of arsenic for certain aquatic organisms. Amer. Fish. Soc. Trans. 61: 226.

(*211*) Surber, E. W., and T. O. Thatcher. 1963. Laboratory studies of the effects of alkyl benzene sulfonate (ABS) on aquatic invertebrates. Amer. Fish. Soc., Trans. 92(2): 152–160.

(*212*) Swisher, R. D., J. T. O'Rourke, and H. D. Tomlinson. 1954. Fish bioassays of linear alkylate sulfonates (LAS) and intermediate biodegradation products. J. Amer. Oil Chem. Soc. 41(11): 746.

(*213*) Tarzwell, C. M. 1957. Water quality criteria for aquatic life. pp. 246–272. *In:* Biological problems in water pollution. U.S. Department of Health, Education, and Welfare, Robert A. Taft, Sanitary Engineering Center, Cincinnati, Ohio.

(*214*) Tester, J. W. 1963. The influence of sodium chloride on the growth and reproduction of sago pondweed (*Potomogeton pectinatus* L.). M. S. Thesis, Utah State Univ. 73 pp.

(*215*) Thatcher, T. O. 1966. The comparative lethal toxicity of a mixture of hard ABS detergent products to eleven species of fishes. Int. J. Air Water Poll. 10: 585–590.

(*216*) Thatcher, T. O., and J. F. Santner. 1967. Acute toxicity of LAS to various fish species. 21st Purdue Ind. Waste Conf. Eng. Bull. Purdue Univ. No. 121.

(*217*) Trama, F. B. 1954. The acute toxicity of copper to the common bluegill (*Lepomis macrochirus* Rafinesque). Notulae Naturae No. 257.

(*218*) Turnbull, H., J. G. DeMann, and R. F. Weston. 1954. Toxicity of various refinery materials to freshwater fish. Symposium on waste disposal in the petroleum industry. Ind. Eng. Chem. 46: 324.

(*219*) Ukeles, R. 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. Appl. Microbiol. 10: 532–537.

(*220*) U.S. Department of Commerce. 1963. Maximum permissible body burdens and maximum permissible concentrations of radionuclides in air and in water for occupational exposure. National Bureau of Standards Handbook 69. Government Printing Office, Washington, D.C. 95 pp.

(*221*) U.S. Department of Health, Education, and Welfare. 1962a. National water quality network annual compilations of data. Oct. 1, 1961–Sept. 30, 1962. PHS Publ. No. 633.

(*222*) U.S. Department of Health, Education, and Welfare. 1962b. Public health service drinking water standards. PHS Publ. No. 956.

(*223*) U.S. Department of Health, Education, and Welfare. 1956. Shellfish sanitation workshop. U.S. Public Health Service. Proc. (mimeo.)

(*224*) U.S. Department of Health, Education, and Welfare. 1958. Shellfish sanitation workshop. U.S. Public Health Service. Proc. (litho.)

(*225*) U.S. Department of Health, Education, and Welfare. 1961. 1961 Shellfish sanitation workshop. U.S. Public Health Service. Proc. (litho.)

(*226*) U.S. Department of Health, Education, and Welfare. 1965a. 1964 Shellfish sanitation workshop. U.S. Public Health Service. Proc. (litho.)

(*227*) U.S. Department of Health, Education, and Welfare. 1965b. National shellfish sanitation program manual of operations. Public Health Service Publ. No. 33. Superintendent of Documents Government Printing Office. Washington, D.C.

(228) U.S. Department of the Interior. 1967a. Pollutional effects of pulp and paper mill wastes in Puget Sound. Federal Water Pollution Control Administration.

(229) U.S. Department of the Interior. 1967b. Temperature and aquatic life. Federal Water Pollution Control Administration.

(230) Veselov, E. A. 1948. The effect of crude oil pollution on fishes. Rybnoe Khoziastvo 12: 21–22. (In Russian.)

(231) Vinogradov, A. P. 1953. The Elementary chemical composition of marine organisms. Sears Foundation, New Haven, Conn.

(232) Wagner, R. 1959. Sand and gravel operations. Fifth Symposium Pacific Northwest on Siltation, Proc. U.S. Public Health Service, Portland, Oreg.

(233) Wallen, I. E. 1951. The direct effect of turbidity on fishes. Bull. Okla. Agr. Mech. Coll., Biol. Ser. 48: 27.

(234) Washington Department of Fisheries. 1944. Annual Report. Olympia, Wash.

(235) Wiebe, A. H. 1935. The effect of crude oil on fresh water fish. Amer. Fish. Soc., Trans. 65: 324–350.

(236) Wilder, D. G. 1952. The relative toxicity of certain metals to lobsters. J. Fish. Res. Board. Canada, 8, 486; Water Poll. Abs. 25: 11, 264.

(237) Williams, L. G., and D. I. Mount. 1965. Influence of zinc on periphytic communities. Amer. J. Bot. 52(1): 26–34.

(238) Wuhrmann, K., and H. Woker. 1948. Experimentelle Untersuchungen über die Ammoniak-und Blausaurevergiftung, Schweiz. Zeits. Hydrologic. 11: 20.

(239) Wuhrmann, K., F. Zehender, and H. Woker. 1947. Über die Fisherei-Biologische Bedentung des Ammonium-und Ammoniakgehaltes Fliessender Gewässer. Vierteliahrsschr. naturfarsch. Gesellsch. Zürich 92: 198.

(240) Wurtz, C. B. 1962. Zinc effects on fresh water mollusks. Nautilus 76(2): 53.

(241) Wurtz, C. B., and C. E. Renn. 1965. Water temperature and aquatic life. Edison Electric Inst. Publ. No. 65–901. 99 pp.

(242) Wurtz-Arlet, J. 1960. Laboratory study of the toxicity of certain synthetic detergents to the rainbow trout. Vortraege Originalfassung Intern. Kongr. Grenz Flaechenaktive Stoffe, 3 Cologne, 3(329), CA 57(10374).

(243) Ziebell, C. D. 1960. Problems associated with spawning and growth of salmonids in Northwest Watersheds. Seventh Symposium on Water Pollution Research, Proc. Pub. Health Service, Portland, Oreg.